LATZER

Bacteria and
Their Activities
In Sewage Waters

BOTANY
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1901

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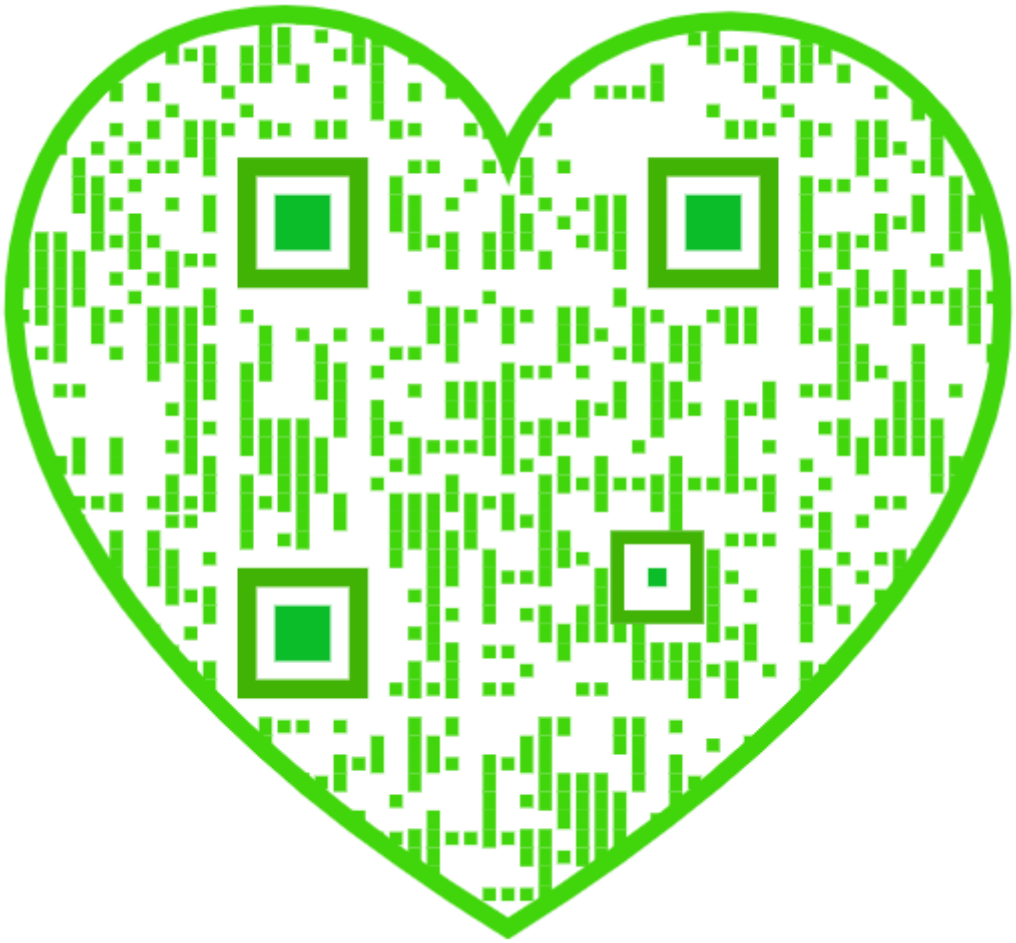

BACTERIA AND THEIR ACTIVITIES IN SEWAGE WATERS

BY

JENNIE MARY LATZER, B. S., 1900

THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Miss Jennie Mary Baker, B.S., 1900

ENTITLED Bacteria and their Activities in Sewage Waters

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The object of this work was to find out the different species of bacteria present in sewage water, the approximate numbers of each, and as far as possible, the character of the special activities of each kind in the destruction of organic matter.

For this purpose the waters for analysis, both bacteriological and chemical have been collected from the septic tank, north-east of Urbana, Illinois. This receives the sewage of about 3500 inhabitants of Champaign, a city in Champaign County, Illinois, having a population of 9700. A general separation of the different micro-organisms was made by plate cultures and these were identified as far as possible, and among them were marked out the most apparent sewage bacteria. The gases produced by the individuals of a given species in glucose broth were analyzed and the changes chemically produced when grown in sterile sewage were determined.

It is needless to say, that bacteria are found in sewage waters where a large quantity of organic matter is present. They occur in immense quantities, as is shown in table I.; as many as sixteen million per cubic centimeter have been found in one case. The numbers are large and there is an extensive representation of species including aërobic and facultative anaërobic kinds. Undoubtably the list as made out does not include all the different species present, but probably the most prominent ones are represented as each of them seemed to have occurred more than once in the cultures.

When sewage , as it frequently does, gets access to drinking waters, through leakage, or other imperfect methods of sewage drainage, it pollutes the water and may contaminate it with disease germs, when such are present in the sewage. This is the history of typhoid fever as recorded by numerous investigators. Pathogenic species are not normal inhabitants of such waters, they have to struggle for survival. The keenness of competition among the dense crowds of saphrophytes makes their continued existence almost impossible.

Sewage as it occurs in practice contains a large quantity of matter other than that productive of disease and it is just upon this comparatively harmless, but constantly present material that the chemists rely for the indications upon which they base their opinions of potable waters. They are unable to say whether or not a sewage-laden water is disease-bearing on any particular date. To them, all sewage is alike, but they condemn the water for the reason, that although it may be harmless to-day it is impossible to predict what may be its conditions to-morrow. The discovery of disease germs in water is only possible after the water has become infected and it remains for the bacteriologists to make this decision.

The distribution of bacteria is very wide. They occur in the air, in waste produce, in the alimentary canal, and in almost every conceivable place, from which they can readily be brought into the sewer.

Both the chemical and bacteriological quality of the sewage depends upon the size of the community, trade-waste, the amount

of rainfall, and other physical conditions. Thus sewers carry water fouled with organic wastes from the human system, from various cleansing processes common to all households and manufactoring wastes. Decomposable organic matter affords an almost ideal nidus for micro-organisms in which the only hinderance to their unlimited multiplication is the presence of their own large numbers. This makes the struggle for existence exceptionally keen. Sewers also carry a large number of these minute forms of life which are obtained from the soil and which have been brought in by the water from the surface after a rain.

Food for bacteria in order to be available must be in solution, for it must pass through the cell wall by osmosis. They do sometimes live on solid material, but in such cases they are able to produce enough chemical change to bring the material into solution to support themselves. Their food must contain nitrogen, carbon, oxygen, hydrogen and small quantities of mineral matter. The carbon and nitrogen are made use of when in the organic rather than when in the inorganic conditions. In sewage these essential elements are present in large quantities, so that there is sufficient food for these organisms. There is constantly a new supply of food brought along which takes the place of that which is consumed.

The life of sewage bacteria depend s on the decomposition of complex compounds into simpler ones accompanied by the liberation of energy to be used in various ways. Micro-organisms when surrounded by the conditions favorable to their growth, - a proper food supply, moisture, favorable atmospheric conditions, and tem-

perature, - develops very rapidly for a time. After a certain period the rate of development diminishes until it finally ceases entirely, although there may be a large supply of food material still unused and the general conditions are not changed. In acetic fermentation there comes a time when an excess of acid inhibits the farther growth of the organism. This is when the acetic acid has approximately reached a percentage of 14. Most food-stuffs are insoluble in water and therefore can not be absorbed by living organisms except as made soluble by the agents called enzymes, which have the power of dissolving or digesting these materials. This action is a very weak decomposition. The enzymes are unorganized ferments. Organized ferments are living organisms and the fermentation which they cause is more profound than that caused by enzymes. Some species ferment one food-stuff, some another, some in acid, others in alkaline media. As living organisms their work of fermentation is equivalent to the entire action of an animal and their products are equivalent to the waste of animal life.

The three principal activities of organized ferments are (1). fermentation of carbonaceous material, such as changing sugars into alcohol and carbonic acid, alcohol into acetic acid, milk sugar into lactic acid, etc.; (2). putrefaction of dead animal or vegetable matter under favorable conditions, when attacked by germs which dissolve and decompose it, often producing bad odors; (3). pathogenic fermentations in which the disease germs produce a toxin.

The process through which bodies are split up by bacteria depends upon the chemical nature of the bodies involved and on the

varieties of bacteria. The destruction of albuminous bodies which is common in putrefaction can be done by whole groups of different varieties, and the action is analogous to what takes place when albuminous bodies are subjected to ordinary intestinal digestion, with the production of peptones. During their growth there is not unfrequently an abundant production of gases. Many of the results of bacterial action depend upon the production by them of unorganized ferments of a very varied nature and complicated action. Such ferments as invert sugars, coagulate casein, split up urea into ammonia carbonate $(NH_4)_2CO_3$ have all been isolated from the products of different bacteria. Some may be diffused into the surrounding fluid and others be retained in the cells where they are formed.

Organic matter in solution seldom oxidizes directly into its final products, but passes through intermediate conditions; the more complex organic forms are resolved into others of simpler structure and these are subsequently burnt up to the stable oxidized compounds, water and carbon-dioxide. These phenomena have been desiginated by the name bacteriolysis. When the action is affected by chemical agents, the term hydrolysis has been used. Other cases in which the decomposition takes place without any absorption of water, may be grouped under the general term fermentation. The organic matter in some cases is partially converted into oxidized compounds, in which the oxygen is not derived from the air but from that originally present in the organic matter or water taking part in the reaction. The resulting products depend upon the kinds of bacteria at work.

From sewage there is an evolution of gases commonly called sewer gas. But there is no specific and definite sewer gas for which a chemical formula of combinations of symbols can be laid down. Dr. Billings says "The air of an ordinary modern, fairly well constructed and ventilated sewer, appears to differ from the street air chiefly in having a higher proportion of Carbon dioxide". He also says that "Specific pathogenic micro-organisms have not been found in air of sewers".

Of the gases found in sewer tanks the carbon dioxide and ammonia is easily dissolved in water, while the hyrodgen from its diffusibility escapes from the tanks more rapidly than do the heavier gases.

Another important change necessary for complete destruction of the organic matter involves the presence of oxygen, either from the air or from the oxidized compound s. Anaerobic fermentations are called by the Germans true putrefactions, faulniss, while aerobic fermentations are termed mouldering, verwesung. In these tanks where the oxidizing agents are eliminated and only the putrefactive ones live and work, the carbonaceous matter is more quickly attacked by them with the evolution of carbonic acid gas and marsh gas. The theory of the septic tanks is that the bacteria living in it are those which can thrive without the presence of air and hence, can cause putrefaction. Those that do the largest proportion of the work live on the sides, bottom and top of the tank where the organic matter collects.

he process supposed to take place in septic tanks

holding sewer water, as described by Ridial" Rideal. Sewage " 74 is as follows: " When the fecal and other solid matters are discharged the earlier stages must be aërobic because of the free oxygen dissolved in the water and that contained in the air. The organisms acting in a normal manner upon these simpler constituents like ammonia, which must obviously exist in small quantities and into which the process itself afterwards dissolves the main ingredients of the sewage. Nitrates in small quantities are consequently often observed in discharges which are moderately fresh. When the free oxygen has been exhausted the bacteria which require air in part diaappears and in part remains quiescent to resume their functions at a later stage. The anërobes will now commence to multiply,- nitrates will be reduced to nitrogen and the liquefaction and hydrolytic changes will proceed and the anöerobic stage of the treatment proper commences. There should be enough oxygen for ammonia and carbonic acid formed with the help of some anäerobic forms and with more oxygen get rid of the remaining products". A large number of these changes most of them hydrolytic are accomplished by enzymes, which are powerful resolving agents. When there is a constant renewal of the material in the tanks, it seems that these actions all have to take place at the same time. Just what species take part in the first step and which in the next is a difficult problem It is very probable, however, that the facultative anaerobic gas producers are the most characteristic of the sewer bacteria.

IV. What is to be done with such a large amount of fouled water? Either it must be (1) turned into a body of water so large as to dilute it beyond all possibilities of offence and where it cannot endanger human life by polluting a public water supply, or, (2) it must in some manner be pruified. In places where such a volume of water is not accessible the latter must be followed.

Sewage purifaction of to-day is as yet in an experimental stage. We know to a certainty how sewage may be rendered harmless. Further knowledge will probably be in the line of making present processes do more work without additional cost. Ordinary sewage stronger even than usual would contain as much as 998 parts of pure water per 1000 c.c. It is only the two parts of of a thousand that causes the trouble. In nature organic matter and bacteria always accompany each other; the removal of one means that the other must in some degree go likewise, and the harmful ones go with the harmless. With the removal of all the organic matter all the bacterial food is gone and without food death comes swiftly even though the bacteria be legions. Several methods of purification have been experimented with and put into actual use. The principles of filtration, precipitation, and sedimentation are applied in all cases. Filtration through several beds of filtering material such as sands and coke are abundantly used. It is an easy but slow process in cases where there is much sewage to be disposed of, if the filter beds are fine enough to remove a considerable portion of organic matter. But where thereis a large amount of suspended material, filters are easily clogged and the filtering process is checked.

There is a varying composition of the sewage due to the length of time elapsing between the passage of the sewage into the sewers and the time of its analysis. From experiments it has been found that there is a loss of carbonaceous matter as shown by the difference in the determination of the oxygen consumed by fresh and stale sewage. When passed through a series of filters it has been shown that there is in the effluent one part more of free ammonia and a half part less of albuminoid ammonia than the sewage contained before filtrations. Stale sewage contains not much morethan half as much organic matter as the fresh. In the report of the Massachusetts State Board of Health for 1897 there are records of experiments carried on regarding the action ofcoli-communis. The river water contains this germ in large numbers; this water is filtered and stored in reservoirs. Four hundred samples were collected at the station just as the water is pumped from the filter to the reservoir, and in only six cases was the presence of Bacillus coli-communis detected. Settling tamks are used to a large extent, in which the sewage is collected until the tamk is filled. It is then allowed to remain undisturbed for a period of two hours and possibly more, depending upon the length of time required to draw off the supernatant liquid. The efficiency of these tanks in removing the sludge from the sewage, and preventing trouble where it is led, depends upon the frequency of its removal, the care exercised to avoid drawing off any sludge with the supernatant liquid.

Purification of sewage by irrigation is another form of filtration. Thesewage is led on a piece of land and afterpassing through a certain depth of soil, the effluent is drained into a

running stream. Other filtering media besides soils and sands, so commonly employed for the purpose are iron, sponge, brick-dust, coke, powdered glass, and many others. They are all used in a very fine state of division; but usually are employed for domestic purposes only.

By precipitation it is quite impossible to obtain effluents which favorably compare in organic purity with these obtained by intermittent filterations through sands, but the processes are often useful in practical work. There are three different kinds of precipitation: (1) in which the precipitant is in a solid and insoluble form, such as sand, clay, (2) in which the precipitatnt is soluble and more or less chemical active, but which upon entering into reaction with the constituents of the water is converted into an insoluble form. In this case there is a little or no bactericidal effect. The materials used are such as lime, causing temporary hardness, and alum when used in small quantities (3) When the precipitant is soluble, chemically active and produces a bactericidal effect in addition to the mechanical one. The latter is used exclusively for sewage, and the chemicals are lime, ferric oxide, alum, and a few others. It has been found by experiments of the Massachusetts State Board of Health, 1889-90, that the results of treatment of sewage with equal money-values of the three substances named are practically alike and that each produce a reduction of about 93 % of the number of organisms.

The septic tank is one of recent inventions for sewage purification. A more extended description of this is given below.

The Champaign Septic Tank.

The following is taken from the article by Prof. Talbot appearing in the Engineering News. Aug. 17, 1899.

The sewage system of Champaign includes 24 miles of sanitary sewers, amounting to, considering all the conditions, probably that of a town of 3500, with all houses connected with the sewer. The mount of water consumed cannot be used as a basis of estimate of sewage flow, since the water company supplies Urbana as well. During dry weather gaugings of the outlet sewer show a flo w of 300,000 gallons per 24 hours, while at times when considerable ground water reaches the sewer the flow frequently reaches several times the above amount. Theoutlet sewer extends for two miles through the city of Urbana, and by reason of the distance and the flat garde over three hours is required for t he sewage from the main part of chamoaign to reach the Tank. The effluent is discharged into Salt Fork Creek.

The septic tank is about 37 feet long, 16 feet wide, and h a depth of 5 feet of water giving a capacity of 22,000 gallons . The tank is enclosed in a brick building having a shi ngle roof. Wooden doors and windows and allow access and light for inspection and cleaning. A longitudinal wall divides the tank into two compartments. While usually both are in operation, one may be kept in use while the other is be ing cleaned. The flow is divided at the valve chamber, and the enlarged mouth of the tank serves to distribute the flow over its full width, and over a considerable depth. Baffle boards on ~~terps~~ are placed across the tank reaching down to a depth of 2 - 3 feet below the surface. These serve to retain floating

matter and to prevent surface currents. The middle one is of concrete, and serves also to stiffen the walls and support the pump. On the end wall at the lower end of the tank, a 6 x 6 inch angle is placed with its upper edge level forming a weir over which the effluenct flows into the outlet channel. The weir takes the water uniformly from the width of the tank, and the depth of flow does not vary more than one inch, thus keeping the tank in a nearly constant condition of flow. The sludge is removed by a 5 H.P. steam engine.

Gas is given off from the tank in considerable quantities and when the sludge is stirred its evolution is quite noticeable. Where a lighted match is applied, a hot flame rises to a height of 3 feet. The analysis of this gas, made by Dr. A. W. Palmer is given later.

Under the principles of darkness ill-ventilation and moderate heat the anaerobic and facultative anaerobic bacteria develop and produce a chemical decomposition of the retained matter (organic) of the sewage, a reduction of its compounds into parts; a part passing off in the form of gases, a part as inorganic matter with the effluent, and part remains as sludge in the tank. The process is continuous and self-regulating and with the exception of the removal of the sludge no labor or attendance is required. The effluent leaving the tank is clear and nearly all of the putrescible organic matter is removed.

It is well understood that by a mere examination of the characters of plate growths, a species cannot be identified. Among a hundred colonies on a plate there may be little or no difference in

in their appearance, but cultures from the different ones may give very distinct reactions. In order to make distinctions between them, growths on as many as twelve different media are made with the characteristics as to their behavior in each observed.

A list of the media used in this work, together with the composition of each will aid in comprehending the descriptions. In all cases the Leibig's beef extract was used instead of meat and the different media were made 10 cubic centimeters of normal acid per litre from the neutral point, with phenolpthalin as an indicator.

(1) Bouillon:-

Beef extract ----------5.0 grams.
Peptone ---------- 10 grams.
Distilled water ----------1000 c.c.

(2) Agar agar:-

Beef extract ---------- 5 grams
Peptone ---------- 10 grams.
Distilled water ----------1000 c.c.
Boiled and filtered.
Agar ---------- 15 grams.
Dissolved in 500 c.c. of distilled water.
Added to first and boiled to 1 litre.
Cleared with egg, strained and filtered.

(3) Glucose agar:-

Same as (2) but after boiling add 20 grams of glucose.

(4) Gelatin:-

Extract ---------- 5 grams.

Peptone ---------- 10 grams.

Distilled water ----------1000 c.c.

Boil.

Gelatin ----------120 grams.

Boil until dissolved, clear with egg and strain.

(5) Glucose gelatin:-

Same as (4) - after boiling add 20 grams of glucose.

(6) Lactose litmus gelatin:-

Same as (4), then add litmus until distinct neutral color is formed, and add lactose --20 grams.

(7) Carbolized litmus broth: (Wurtz).

To (1) add litmus and carbolic acid 1.15 C.C.

lactose ----------20 grams.

(8) Milk:

Ordinary milk, skimmed with separator.

(9) Litmus milk:-

Same as (8) with the addition of litmus.

(10) Glucose broth:-

Same as (1) and

Add glucose ----------20 grams.

Fill in fermentation tubes.

Sterilize in autoclave without pressure.

(11) Sugar free broth:-

Same as (1).

Inculate with a culture that forms gas in order to

remove all the sugar present for from 14-18 hrs.

Clear with egg.

(12) Nitrite solution:-

Peptone ---------- 1 grams.

KNO_3 ---------- 2 grams.

Distilled water(Free from ammonia) 1000 c.c.

(13) Sewer gelatin:-

Sewer water : 1000 c. c.

Gelatin ---------- ---120 grams.

Boil.

Titrate.

(14) Sewer-bouillon-gelatin.

Sewer water ---------- 500 c.c.

Bouillon ---------- 500 c.c.

Gelatin ---------- 120 grams.

(15) Potato:-

Boil potato until soft, peal with sterile knife - cut in slices and sterilize for 30 minutes or,

Cut while raw with cork borer and divide diagonally soak in water over night, fill tubes and sterilize for 30 minutes.

VII. The water used for the colony counts was collected in 4 oz. ground glass stoppered bottles and covered with a piece of oil cloth around the neck and stopper. These bottles were previously washed and sterilized in hot air sterilizer at 160 degrees for one hour. The collection was made by quickly opening the stopper and filling the bottle with the water up to an inch from the neck.

Counts were made from waters taken from three different parts of the tank. The water designated "top" was taken fron the second section and from just beneath the surface, this water was usually quite clear, but with heavy suspended particles. That designated by "middle" was the water taken from the first section at a depth of about two feet. This was always black with gas bubbles rising to the surface and giving off a very bad odor.

The "effluent" was taken from the stream leading from the tank. After the collections were made and the temperature of the water taken the bottles were closed tightly and immediately brought to the laboratory. Plating was begun in every instance within two hours after the arrival of the bottles. Since there are as many as a million bacteria in a cubic centimeter of this water, dilutions had to be made. It was found by experiment that a dilution of 1 - 10,000 resulted in a countable number of colonies on each plate. This dilution was made in flasks containing 99 cubic centimeters of sterile water. The flasks were filled with 102 cubic centimeters of distilled water, allowing 3 cubic centimeters for evaporation, during sterilization. Into this flask was added exactly one cubic centimeter of the water to be analyzed. The flask was well shaken and again one cubic centimeter of this water transferred to a 99 cubic centimeters of sterile water in another flask. After thorough shaking one c.c. of this second flask was taken and added to a tube containing melted agar; with this a plate was made and allowed to stand six days before the counts were made.

The number per cubic centimeters obtained in thisway

is only an approximate number, as in the process there is a large field for errors. In the first place bacteria exist in water as solid particles in suspension and it can not be assumed that there is an equal distribution, then there are or are liable to be errors in measuring the quantity of sewer water, a single drop of which would make a large difference. Many bacteria will remain adhering to the sides of the glass and again after pouring some remain behind with the agar., smearing the tube. Not all bacteria develop under conditions surrounding them in the media employed. Some grow slower than others and may be obscured by some spreading colony, while others may not thrive at all in the medium. which is employed etc. The number of course vary with the time they are kept before counting, but it is supposed that in from 5 to 6 days all o - dinary water-bacteria would develop. There is also a possible error which may arise from being admitted through other sources.

The plates were kept at room temperature during the time of growth, a few though were maintained at 37 degrees Centigrade. This latter temperatureis supposed to kill all ordinary water-bacteria, so these plates would give the relative number of sewage bacteria. Anaerobic cultures were also tried but without great success. Illuminating gas was used for displacing the air but this was found later to contain .6 per cent of oxygen. In the results given of these tests some aërobic species may be included.
Then the gas was passed through pyrogallic acid to dissolve out the oxygen and a dish containing the same substance was placed inside the vessel to complete the absorption of any remaining oxygen.

In every case that this was tried the plates were dried so much that no colonies appeared. Another method was used by passing the gas through the acid before entering the jar and using a large basin of water on the inside to keep the media moist. This gave some fair results with only a few colonies appearing on the plates. When these plates were placed so that oxygen could be admitted without any exposures, numerous colonies appeared, showing that the growth of the obligatory aërobic had been inhibited during that time.

It is very difficult and in some cases impossible to institute comparisons between samples of the raw sewage and the effluent, since the composition and the number of bacteria present are continually varying at different times of the day. Even the samples collected at the same time would not give a conclusive test, since there may be a complete change in quality during the time it takes the effluent to pass from the first to the last stage.

Tests for Bacillus coli-communis were made and in all cases examined positive results were obtained. For coli determinations some of the water is added to a tube containing the Wurtz broth, this after one day shows a red color with gas bubbles collecting on the surface. Lactose-litimus gelatin plates were made from this. After two or three days the coli-colonies when present will change the plate red and it can be distinguished from the other colonies by its peculiar fan-shaped appearance. Subcultures are now made from the plate colony in milk, sugar free broth, and the fermentation tube. If present it will coagulate milk form indol and produced about 35 per cent. of gas in the fermentation tube.

The following is a table showing the approximate number per cubic centimeter found in the waters at different times. Each number is the average of several plates.

Table 1.

Date	"Top" Temp	Serial No.	B. Coli-communis	Aërobic colonies thousands per c.c.	Anaërobic colonies thousands per c.c.	Thousands per c.c.
Oct. 2.	--	10	+	260	290	
Oct. 6	--	12	+	600		
--- 9	--	14	+	880	235	
--- 15	--	16	+		350	
--- 23	--	20	+	600	340	
--- 30	--	24	+	1040	375	
Nov. 5	--	28		870	103	
--- 13	15	30	+	4650	725	
--- 20	15°	34	+	235	293	
--- 27	14°	36	+	220	53	
Dec. 11	14°	40	+	205	80	
Jan. 8.	12°	44	+	1385	65	
Jan. 15	11°	54	+	250	47	23
--- 22	10	68		90	70	80
--- 29	10	80		200	25	50
Feb. 13.	10°	98		360	20	

Table II.

"Middle"

Date	Temp-erature.	Serial No.	B. Coli-communis	Aërobic colonies thousands per c.c.	Anaërobic colonies thousands per c.c.	Thous-ands per c.c.
Oct. 2.	--	11		470	840	
--- 6	--	13	+	4103		
--- 9	--	15	+	1550	430	
--- 15	--	17	+	1550		
--- 23	--	21	+	240	257	
--- 30	--	25	+	4040	900	
Nov. 5	--	------	---------	----------		
--- 13	~~15~~	31	+	2400	2040	
--- 20	--	--	----	- ---	---	
--- 24	14	~~37~~	+	16330	4120	
Dec. 11	14	41	+	155	30	
Jan. 8	12	46	+	950	50	
--- 15	12	55	+		480	60
Jan. 22	12	71		1930	530	490
--- 29	10	82		860	360	250
Feb. 13	10	100		1480	0	
"Effluent."						
Jan. 29	10	84	+	30	3	10
Feb. 13	10	102	+	105	0	

There seems to be no general increase or decrease as regarding temperature, nor are the increases and decreases at regular intervals. From the table it will be seen that the number from the middle varies from 155,000-16,330,000 and give a general average of 2,696,000 per cubic centimeter. The number grown with the incomplete anaerolic method, cutting off most of the obligatory aerobes range from 30,000 to 4,120,000 per C.C. an average of 914,000. Approximately one fourth of the number in the cases examined are regular sewage bacteria. In the waters from the "top" the number varies from 90,000 to 4,650,000 per cubic centimeter, an average of 794,000. By the anaerobic method used the number varied from 20,000 to 725,000 an average of 206,000. Of the three cases tested for sewage bacteria there were found two-sevenths of the whole number belonging to this class.

According to the number found in the effluent as compared with the "middle" and "top" we can conclude that only one fourth of those occuring in the "top" and one seventeenth of those in the"middle" remain in the water leaving the tank.

Owing to the fact that bacteria are dependent for the most part on previously prepared nourishment, it seems quite probable that those obtained directly from water are degraded forms. For this reason Fuller has suggested that in order to bring them to more similar conditions, they be grown first on beef broth for three days, then on gelatin plate, from which after three days they may be transferred to an agar slant, and from this all subcultures should be made. This was carried out for species I to XX inclusive and from XXXV to XLII inclusive. In order to economize time, in species XXI to XXXV

the agar slants were made directly from the colonies on the plate, and all the subcultures from the slants.

The degree of reaction which is most favorable for bacterial growth varies somewhat with different species, and with bacteria of different degrees of vitality but of the same species. To this are due the differences in reaction of the same species by different experimenters when in the different cases they are planted at different states of vitality. The following is a table which shows the degree of vitality to some extent of Bacillus vulgaris.

TABLE III.

Different degrees of vitality of B. vulgaris.

	Broth turbid.	nitrates reduced.	Indol.	Milk coag.	Gel. liq.	Gas.
A.	–					
B.	+	+	+	+	–	+
C.	+	–	+	+x	–*	+
D.	+	+	o'	+	–*	+
E.	+	o	o	+	–	–
F.	+	–	+	+	–	+
After repeated transplantations.						
B.	+	+	+	+	–	+
C.	+	+	+	+	–	+
D.	+	+	+	+	–	+
E.	+	+	o	+	–	+
F.	+	+	+	+	–	+

'o indicates slight action.

x coagulated after boiling.

* a membraneous growth around growth along the path.

This very common form, B. vulgaris, occuring so abundantly, forms colonies which are not identical, yet there is some similarity which distinguishes it. Six of these colonies, all appearing on different plates but from water collected at the same time, were taken, subcultures were made and tests made as is shown with results in the preceding table. As is shown there occurs to be a different degree of vitality which after repeated transplantations become more uniform.

Plates of the above colonies were made on plain gelatin, sewer gelatin and broth-sewer gelatin, in which the following results were obtained: B.C.D.E. grew best in the broth-sewer gelatin while F. on the plain gelatin; the colonies were larger and seemed more vigorous than those on the other plates. Subcultures from each of three plates were made, but no difference in vitality of the three taken from the same original colony became evident.

No classification of Schizomycetes has yet been made that has been universally adopted; the following one as given by Migula was followed in this work.

I. Cells spherical in the free state, not elongated before division. Cell division in one, two or three planes. Coccaceae.

A. Cells without cilia (not motile).

a. Division in one plane.....Streptococcus.

b. Division in two planes.....Micrococcus.

C. Division in three planes.....Sarciua.

B. Cells with cilia (motile)

a. Division in two planes.....Planoccus.

b. Division in three planes.....Planosarcina.

II. Cells cylindrical division in one plane only and elongate to the double length before division.

A. Cells straight, rod forming without slant motile or non-motile.

.....Bacteriaceae.

a. Cells not motile.....Bacterium.

b. Cells motile (cilia).

1. Cilia distributed over the entire body....Bacillus.

2. Cilia polar.....Pseudomonas.

B. Cells bent, without sheath.....Spirillaceae.

C. Cells surrounded by a sheath.....Chlamydobactiriaceae

D. Cells without sheath in threads, motile by means of an undulating membrane.....Beggiatoaceae.

Following are the descriptions of all the species of bacteria worked out during the course of these investigations. Those species for which a name could not be found are designated by Roman numerals only. Of the forty-two species described it is quite evident that the gas producing ones are most active, of these however the ones appearing most frequently were species numbers II, III, IV, VII, X, XII, XXVIII, XXIX, XXIII, XLI; some of these occuring on every plate. Of the non-gas producing ones XIV, XVII, XXIII, XIX, XXIII appear to be the most active. As will be shown later it is probably not one specific kind that produces any great change, but that it is the many acting together that do the great work. Following the descriptions of the various kinds is given a condensed table showing at a glance the characteristic behavior of each one in the various cultures. The plus and minus signs are used to denote the presence or

absence of the character named at the head of the column.

I. BACILLUS SUBTILUS (Ehrenberg).

It is a long, slender bacillus, varying in length from 2 - 8 u. being from 3 - 6 times as long as wide. They occur single on agar slants but in broth grow out in long filaments. It is actively motile. Spores are formed at or near the middle of the rod. It grows best at blood heat(incubator at 37° C.), but also at the room temperature on ordinary media.

Plate Colony: A grayish white, wrinkled colony, spreading, dull surface. On gelatin it forms a saucer shaped growth containing a central opaque portion from which radiate grayish, granular and branched growths, a regular net-work which extends to the circumference of the liquid part.

Agar Slant: A grayish white, dull, wrinkled growth, spreading; extending over almost the entire surface. Coarse thread-like granular projections, radiating into the media from several different points.

Gelatin Stab: Liquefaction commences in the shape of a membraneous sack including a gas bubble at the surface and slight sediment in the bottom. Later this extends from side to side of the tube until in about a week entire contents is liquified with heavy sediment at bottom and clear liquid.

Milk: Coagulated and in four days had liquified the casein.

Litmus Test: It is not acid in reaction.

Fermentation Tube: No gas, no growth in closed arm, but heavy growth in open bulb.

Indol: No indol is produced.

Reduction Test: Nitrates are not reduced.

Bouillon: Liquid limpid, with a white wrinkled pellicle extending up the sides of the tube.

Potato: A grayish white growth, very abundant, later becoming wrinkled.

Remarks: It has occurred on several plates, and this extremely common bacillus might not be considered as one of the active sewage bacteria.

II. BACTERIUM UBIQUITUS(Jordon).

It varies in different cultures tending to form filaments in broth. It is a short almost oval bacillus length equals 1 1/2-3 times width. Ends are curved, non-motile; spores were not observed. It grows on ordinary culture media at room temperature, also at 37° C.

Plate Colony: At first a small round shiny, white colonies, some with dark centers others none, and the deep colonies are dense and oval. Later they increase in size, have well defined and shaped outline, through the microscope they are densely granular.

Agar Slant. Aftertwenty-four hours is in form of small round smooth, shiny white colonies which soon run together and spread over almost the entire surface.

Gelatin Stab: Growth along path of needle is in form of separate colonies of different sizes, with a small white growth on surface. Later gas bubbles are formed across the growth. It does not liquify the gelatin.

Milk: After three days, it has coagulated the entire contents.

Litmus Test:Turned litmus red after two days.

Fermentation: Gas is found in the closed arm, liquid turbid in both arms.

Indol: Indol is formed in sugar free bouillon.

Redustion Test: It reduces nitrates to nitrites.

Bouillon: The liquid becomes turbid with a white sediment, a very thin almost transparent pellicle is formed, which upon slight shaking falls to the bottom.

Potato: It forms a heavy beaded, raised yellowish growth with brownish tinge. It is not spreading, but is luxuriant.

Rosalinic Acid:This acid is slightly faded after a great length of time.

Remarks: This is found in Sternberg's Manual as bacillus ubiquitum. It is found frequently and seems to be one of the active members.

III. BACILLUS COLI-COMMUNIS(Escherich).

It is a short bacillus, being about 1.4 u. broad and from 1 - 2 times as long. Found single and not in filaments, actively motile; observed no spores, it grows best at 37° C.; also grows at room temperature.

Plate Colony: It is a small opaque colony, slightly spreading. On litmus gelatin plate it has most frequently a fan-shaped appearance. Always on the surface of the media, for it does not grow with the absence of oxygen. Not all varieties have this shape, others are round and smooth.

Agar Slant: It forms a grayish growth along path, later spreading. surface quite rough but is shiny. Edges are irregular.

Gelatin Stab: Growth is slightly granular along the tract, with a heavy and wide spreading surface growth, reaching the sides of the tube.

Milk:Milk is coagulated at the end of two days.

Litmus: It gives an acid reaction.

Fermentation: It is a good gas producer filling about one-half of the closed arm.

Sugar Free Broth: After three days it produces indol. Some cases only a trace is formed.

Bouillon: . This becomes turbid with a white sediment and a very thin, membraneous pellicle, which falls to the bottom upon slight shaking.

Remarks:This has been found in every sample collected, and in all parts. Sometmes however, there was only a very faint indol test.

IV. BACTERIUM CAUALIS (Mori).

It is very small and appears much like a micrococcus. In broth they are larger and here about one and one half times the width, mostly in pairs, some are slightly motile, observed no spores.

Plate Colony. It is a soft grayish mass, shiny and uniform, slightly spreading when the edges are lobed. On gelatin it is smaller and more compact.

Agar Slant:Growth along the path and spreading on both sides, uniform, slightly decreasing in width towards the top.

As it gets older it spreads laterally and the edges seem as though it broke, giving it a serrate edge.

Gelatin:Stab: Only a slight membraneous growth along the path, but a small grayish white growth at point of innoculation, giving it a nail-shaped appearance.

Milk: The milk coagulated at the end of two days.

Litmus: An acid reaction.

Fermentation: Gas is formed with growth both in closed and open arm.

Indol. A faint indol test is obtained.

Reduction: It has not the power to reduce nitrates.

Bouillon: The liquid becomes turbid with white sediment at the bottom of the tube. A ring is formed on the glass at the top of the liquid but no pellicle.

Potato: At first the growth is the color of the potato, later it is turned brownish. It becomes heavier after several days and is slightly spreading.

Rosalinic Acid: It does not decolorize it.

Remarks: This corresponds closely to the description by Migula P.351 only that in the specimen I examined, I observed no capsule, nor was any pellicle apparent, yet the ring was very prominent so that there may have been a pellicle formed and fallen.

V. BACILLUS MYCOIDES(Flügge).A large organism, the length being equal to 2-3 times the width. Ends square, occurs in long filaments. It is actively motile. Spores are formed near the ends of the rods.

It grows in temperature of 15° - 37° C.

Plate Colony: It is a large gray mass of inter-woven threads, spreading radially, denser in the center. At first only a dim spot, which gradually develops into this mass and on gelatin this mass remains tender and clear, but as it rises the gelatin is liquified and the growth spreads leaving a dull mass of growth.

Agar Slant: In one day the growth has spread over almost the entire surface, a dull white, fluffy irregular growth. After several days there is a mossy growth extending down into the agar.

Gelatin Stab: At first there is a gas bubble formed at the top and beneath it along the stab there is a growth with thread like root-hairs projecting out laterally from the stab. Soon there is a membraneous sack formed beneath the bubble which gradually enlarges, with contents liquefied. After seven days the gelatin to the end of the stab is liquified and is separated from the non-liquified gelatin by a flocculent growth leaving the top part quite clear.

Milk: It is coagulated at the end of two days, and in four days almost all the casein is liquified.

Litmus: There is no change in color.

Fermentation: No gas is formed but growth in both open and closed arm.

Indol: No indol is formed.

Reduction: It reduces nitrates.

Potato. Growth a dull, white, spreading growth with rather irregular surface.

VI. BACILLUS SUBCOCCOIDES(Weichelbaum).

It is a small bacillus with its length about twice the width. Mostly single, some in pairs. Aërobic. Some are actively motile, while others have only a very slight motion, if any at all, spores were not observed. It grows between 9 - 37° C. but best at the room temperature.

Plate Colony: It has a very characteristic appearance, with its peculiar marlings on the surface colonies. The deep colonies are denser and less irregular in shape. On gelatin it has similar markings with the center slightly raised, smooth edges and granular. On older colonies the small lines are numerous and with deeper forrows, slightly yellowish in color.

Agar Slant: At first the growth is a smooth white shiny growth with separate colonies near the top and bottom they are confluent, the separate colonies have markings similar to those on plate. The middle part of the growth is slightly raised. Later this middle ridge is yellow with a creamish lateral growth.

Gelatin Stab: Very slight growth along part of the needle, with a small creamish growth on the surface.

Milk: Not coagulated, but changed chemically, has peculiar odor.

Litmus: Not changed.

Fermentation Tube: No gas. Growth only in open bulb.

Indol: No indol produced.

Reduction: It does not reduce nitrates.

Bouillon: Turbid with sediment and a thin white pellicle, having the appearance of green on surface of cold water. Later the liquid be-

comes ruddy and has a bad odor.

Potato: Growth visible, a shiny spreading growth of a grayish color. It changes the color of the potato to a bluish gray.

Remarks: This is the species which in Sternberg's Manual is given as aquatilis sulcatus III. It conforms with all the different descriptions that I have found on the species with the exception of Fuller, who says there is no pellicle formed on broth. It has occurred several times although not, as abundantly as some of the others.

VII. BACILLUS URINAE.

It is a very short, oval bacillus mostly in pairs, and some in clusters, motile, spores have been observed. It grows in the usual media at room temperature, also at 37° C.

Plate Colonies: They are at first very small, the deep ones are round, sharp edged, and of a brownish color, those on the surface are more irregular in outline and of a grayish white color. Under the miscroscope they are coarsely grained near center becoming almost transparent at the periphery. Some having a dark nucleus, while others are withoutit.

Slant Agar: At first it is a white smooth growth increasing in size upwards, later it becomes waxy with lobed edges and slightly irridescent.

Gelatin Stab: Not liquefied, a granular growth along path, with surface growth extending over almost the entire surface.

Milk: Coagulated after two days.

Litmus: Turned red.

Fermentation Tube: Gas is formed. About one half of the closed arm is filled. Growth both in closed and open arm.

Indol: No indol is formed.

Reduction: It reduces nitrates.

Bouillon: Liquid turbid with sediment, a pellicle is formed which soon breaks and falls. It has a very bad odor.

Potato: A dirty-yellowish firm growth limited to the path of the needle, edges are slightly lobed and middle raised.

Remarks: It seems to agree very closely with all the reactions given by Migula, but I have not observed any H_2S being given off. It was such a short bacillus that it could hardly be distinguish from a coccus. It is a colony which occurs very frequently, and has very much the appearance as well as the characteristics of No. XXVIII With the exception that this is irridescent and reduces nitrates.

VIII PYOGENES(Passet). A short-jointed bacillus, single, in pairs and some in filaments. The latter are formed in broth, while on the slant they are single. Width equals about .5 - .7 u and the length varies from 2 to 4 times the width. Non-motile. Observed no spores. It grows at a temperature of 10 - 37 ° C. but best at room temperature

Plate Colony: Round, granular, very thin and marked with concentric bands; it has a whitish color and the bands alternate with the dark and light bands. The edge is dentate and lobed but sharply defined.

Agar Slant: A white growth spreading over the entire surface in form of white colonies, smooth and shiny giving a slightly greenish cast to the agar. While the growth has a metallic lustre.

Gelatin Stab: A granular growth along the path, with a surface growth having the concentric rings. It does not liquefy the gelatin.

Milk: It is coagulated.

Litmus: Turns red.

Fermentation Tube: Gas is produced, growth both in closed and open arms.

Indol: Only a faint indol test is obtained.

Reduction: Only slightly reduces nitrates.

Bouillon. Turbid liquid, a pellicle is formed which soons falls and part remains on the surface. Later it clears so that the liquid becomes opalescent with a heavy white sediment.

Potato: A white slimy growth, very abundant, changing the color of the potato. Later it becomes entirely spread over the entire surface and has a slightly pinkish tinge, and becomes slightly wrinkled.

Remarks: I have not succeeded to obtain gas bubbles on potato culture this may be due to a different manner of preparation of the media. The same colony has occurred several times and seems closely related with coli, varies in that this is non-motile and the appearance of the

colony. The bands that seemed to be so marked, may have been bands of different ages of growth. It differs fron Bacillus aerogenus only in the shape of a plate colony, and being not as abundant. It is commonly called pyogenus foetidus.

IX. BACILLUS NEBULOSUS.

It is a small bacillus, length being equal to about 1 1/2 times the width. Many in pairs and some in long filaments; motile; observed no spores. It grows about equally as well at room temperature as it does at 37° C. No growth was obtained from 5 - 10 ° C.

Plate Colony: To the naked eye it is a round colony, with a greenish center, then a brown circle followed by an almost transparent peripheral band. The edges are very sharp and regular. Through the microscope it is granular, yellowish with a heavier lining of the interior part and lighter on the periphery.

Agar Slant: It is a cream-colored rough, surface; slimy, irregular margin and spreading at the base. Later it becomes light brown in color, and turns the agar to a very dark brown color. With age the growth itself becomes reddish brown.

Gelatin Stab: At first there was a membranous growth along the path with a surface colony, this soon began to sunk and enclose gas bubbles. This sack now formed, gradually enlarged and liquified the contents.

Milk: It did not coagulate the milk, but the vasein is liquefied and has a bad odor.

Litmus: Not changed.

Fermentation Tube: No gas; no growth in closed arm.

Indol: None produced.

Reduction: Slightly reduces nitrates.

Bouillon: Liquid slightly turbid, abrownish flaky precipitate suspended, a white pellicle is formed which falls upon slight shaking. Later the liquid becomes quite clear and ruddy.

Potato: There was apparently no growth.

Remarks: This does not agree very well with Nugula's description given for this species, but it does conform with that more lengthy description given by Wright, in memoirs of teh National Academy of Science, P. 461. With the exception that I could not make it form gas with glucose broth. He does not say what kind of sugar he used for this test, but I felt assured that this must be the same species for it closely agreed with the rest of his description. This species was found or noticed only two or three times.

X. BACTERIUM AEROGENES(Escherich(.

It is a very short bacillus, with rounded ends; single but usually in pairs, while in broth they frequently occur in more than twos. Width is equal to about 1 u and length one and one half the width. Facultative anaërobe; non-motile; spores were not observed. There is only a trace of growth at 8 - 15 ° C. It grows

well at room temperature, but is probably better grown at 37°C.

Plate Colony: At first it is a very small colony about the size of a pin's head, later it becomes slightly larger and projects above the surface in a conical shaped way, which with age drops down upon the gelatin. They are grayish white smooth and shiny.

Agar Slant: Grown in the incubator at 37° C. the growth is in colonies and slightly iridescent. When grown at room temperature the growth is in the form of a grayish uniform slightly spreading line, and there is no irridescence; smooth and shiny, edges regular.

Gelatin Stab: A uniform growth along the tract which later becomes roughened by small lateral colonies. There is also a shiny white surface growth at point of innoculation.

Milk: It is coagulated within three days, the casein is not liquified.

Litmus: Turned red.

Fermentation Tube: Quite a large per cent of gas is formed, with growth in both closed and open arm.

Indol: A faint test of indol.

Bouillon: Liquid becomes turbid within twenty four hours, a slight ring on glass and a heavy white sediment. An incomplete pellicle is formed which falls with very slight shaking.

Potato: Growth along the line of innoculation, spreading, shiny surface but not smooth. It is at first the color of the potato which later turns brownish.

Remarks: It is commonly called Bacillus lactis aërogenes . It is one of the most active and ever present bacillus I have made a more extended study of this species, which will be given farther on in

this paper. It is easily recognized from any of the other species by its peculiarity on the gelatin plate. Its reactions are like Bacillus coli communis but differs from it in its morphological characters. The irridescence produced when grown at 37° C. and not when at the room temperature is an example of variation due to environment.

XI. BACILLUS RAMOSUS(Frankland).

It is a large bacillus, the width is about 1.6 u and the length varies, about 4 times as long as broad, ends are rounded; they occur single but mostly in long filaments, when the separate cells are not apparent. The long threads are not motile, but the single ones exhibit a slight pendular motion. Spores are formed in the rods, near the ends, it grows well at room temperature, also at 37° C.

Plate Colony: It is a wide spreading colony, composed of thin branching threads interwoven. On gelatin it is at first a cloudy mass, then spreads from the center which is slightly heavier into the surrounding media in a nest-shaped way and begins to liquefy the surrounding media.

Agar Slant: It is a dull grayish white growth, irregular and spreading over almost entire surface. Later turning the agar to a brownish color.

Gelatin Stab. A first a feathery growth all along the path, then

begins to liquefy in a stocking shaped manner, soon the entire contents are liquefied, with a heavy white pellicle on the surface, liquid is clear.

Milk: Coagulated at the end of two days and litmus milk is decolorized. It also liquefies the casein.

Fermentation Tube: No gas is formed, no growth in closed arm.

Reduction: It strongly reduces nitrates.

Bouillon: At the end of one day a cottony pellicle is formed with liquid perfectly clear. Upon shaking the pellicle falls and a new one is formed.

Remarks: It is not a very common occurrence in this water and is easily recognized by its peculiar plate colony.

XII. BACTERIUM ALBUM. (Migula).

It is a short, small bacillus with rounded ends, usually in pairs some single, a few in chains of four to six. The length of the rods varies from 1.2 - 1.[illegible] u and from .6 - .8 u in width. They are not motile. No spores were observed. It does not grow at a low temperature, best grown in a room at room temperature, also at 37° C. It is a facultative anaerobic.

Plate Colony. To the naked eye it is a round colony bordered by a heavier band and a heavy nucleus. The contents is almost transparent and looks like a drop of grayish liquid on the surface. Through the microscope its edges are irregular heavy granular and lines radiating from the periphery inward almost disappearing as it

reaches the center. The transparent portion is only very finely grained. The colony is almost porcelain white.

Agar Slant: It is a grayish white growth along the path, the middle part is almost transparent, this is lined by a darker margin which when grown in the incubator has a fringy appearance. Here it also has a metallic lustre and slightly irridescent, which is not the case when grown at the room temperature. Here it is a very shiny growth and smooth.

Gelatin Stab: Slight surface growth, with a uniform growth along the path of the needle. Here it is one mass of closely packed colonies. A gas bubble was formed at the top and the surface growth spread itself on the walls of the bubble.

Milk: It is coagulated.

Fermentation: It is a great gas producer for analysis see, P ----90 Growth both in closed and open arm.

Indol: No indol is produced in 3 days.

Reduction: It strongly reduces nitrates.

Potato: A slightly spreading, shiny raised creamish growth.

Bouillon. The liquid becomes very turbid, with no apparent pellicle and a white sediment which later turns brownish.

Remarks: This agrees with Nugula's description given on page 419, as is given by him, I have found, that acid is formed at high temperature and alkaline when grown in the dark at room temperature. I have not calculated the per cent, nor would be liable to agree with his as he has not given the media used the quantity etc., my results ~~as~~ found 5 c.c. Bouillon = 1.1 n/20 c.c. NaOH.

Grown in the dark 5 c.c. Bouillon = .8 n/20 c.c. NaOH.
Grown in the incubator 5 c.c. Bouillon = 1.5 n/20 C/C/ NaOH.
After it has been transplanted a number of times the growth is uniform throughout, shiny and slimy.
Made analysis of the gas produced and the changes produced in the water.

XIII. BACILLUS HYALINUS.(Jordan).

It is a large bacillus 1.5 u wide and length varies being from 1 1/2 - 3 times as long. It is very frequently found in chains of various length, when separate cells can hardly be distinguished. It is a motile organism, both when single and in threads. No spores were observed. It grows well at 37° C. It grows not quite so well at the room temperature.

Plate Colony: A dull foggy mass with a heavy center, having a tendency to have a proteus appearance. Slightly wrinkled and a grayish white in color. Edges of colonies are very irregular and they grow to be more than a centimeter in diameter.

Agar Slant: The growth spreads over the entire surface and is a wrinkled, fluffy, ragged growth of a grayish color. Grows well at the end of 24 hours.

Gelatin Stab: At first it is a membraneous growth all along the path but soon this widens and becomes a sack like injection, wider at the top, where the contents is evaporated. This continues to widen and deepen, until the entire contents of tube is liquified with a

heavy, whitish, brittle growth on surface and on bottom a cottony sediment with liquid clear.

Milk:Milk is coagulated in a few days and liquifies a large part of the casein.

Litmus.- Not turned red, and soon decolorizes litmus milk.

Fermentation tube. No gas is formed.

Indol is produced in three days.

Reduction: Nitrates are reduced.

Bouillon: Liquid becomes turbid, a slight sediment and an incomplete pellicle.

Potato: Only a slight growth on potato, the color of the potato . It is in form of colonies, dull and bristly edges. It soon changes the color of the potato slightly.

Remarks: This is a very common organism in this water, it occurred very frequently especially on the plates cultivated in the incubator. This corresponds to the description given by Migula on Page 724 and there is no doubt that this is the same organism.

XIV. BACILLUS ----------.

It is an actively motile bacillus with rounded ends, width about .5 u length varies greatly from 1 1/2 to 6 times the width. Usually single but also occasionally in long threads. The bacillus stains readily, in the middle of the rod I frequently found an oval non-stained body having very much the appearance of spores.

Plate Colony: On agar it spreads out from the center in long branches, which branch again, the ends of these final branches are heavier like a bead at the ends. It is a grayish white mass. In the incubator it spreads over entire plate. On gelatin it is a whitish cloudy growth spreading, upon reaching the surface it spreads laterally and soon liquefies the surrounding gelatin by sending out fine threads into the media.

Agar Slant: Here is formed a grayish white mass at first only a branchy growth but soon spreading over entire surface. It first forms at the base by branch-like projections . This is soon all grown over and sends out a new supply and continues this process until the entire surface is covered.

Gelatin Stab: Soon a membraneous sack extends half way down the growth along the path, with the contents of the sack liquefied. It soon reaches from side to side and continues downward as far as the stab projected. The liquid part is turbid with a heavy sediment.

Milk: Milk is coagulated and liquifies the casein.

Litmus: Litmus is not changed.

Fermentation Tube: No gas, no growth in closed arm.

Indol: none.

Reduction: It does not reduce nitrates.

Bouillon: liquid, turbid; heavy, granular pellicle and brownish sediment.

Potato: A white slimy layer which later turns yellowish and changes the color of the potato to a bluish gray.

Remarks: This is very common and when it does occur it usually spreads over almost the entire plate.

I could not classify this species according to Migula nor Sternberg. It is undoubtedly a characteristic sewage bacteria. A change as produced on water is given later.

XV. PSEUDOMONAS FLOURESCENS (Flügge).

It is a small rod-shaped bacillus, its width is about .7 u and the length about 2 times the width. They are very motile, usually single, some in pairs and in irregular groups. It is aërobic and grows best at the room temperature. No spores.

Plate Colonies: It is a grayish white colony giving a green fluorescence to the surrounding media. It is round, slimy with smooth edgesunder the microscope it is very granular near the center, decreasing towards the periphery, it is of a yellow color fading to almost transparent. On gelatin it soon liquifies, with the bacterial mass collecting at the bottom, and the liquid takes on a greenish-yellow color.

Agar Slant. A smooth shiny, grayish growth, slightly spreading , decreasing towards the top, with sharp edges and giving a green fluorescence to the agar, more so with age.

Gelatin Stab: At first a membraneous growth along the path, which soon begins to liquefy in a funnel-shaped way. Soon the entire contents are liquefied with a brownish sediment and a turbid olive green liquid.

Milk: Milk is coagulated and the casein is soon liquefied.

Litmus: Litmus is not turned red.

Fermentation Tube: No gas is produced.

Indol: Indol is not formed.

Reduction Reduction of nitrates does not take place.

Bouillon: Liquid becomes turbid a whitish sediment and a thin membraneous pellicle is formed on the surface.

Potato: A brownish growth along the path of the needle, which changes the color of the potato to a bluish gray.

Remarks: This occurs quite frequently and can easily be recognized as one of the fluorescent species by its action on the media. But they cannot be recognized from the non-liquifaciens without the test on gelatin. It is commonly called Bacillus fluorescens liquifaciens. Migula places it under Pseudomonas since its motility is caused by polar flagella.

XVI. PSEUDOMONAS PAUSINII.

It is a slender rod, with rounded ends, varying in length from 2 - 4 times its width of the cell. Usually single, but frequently also in pairs, not in chains, non-motile and observed, no spores. Grows best at room temperature, aërobic, and chromogenic.

Plate Colony: It is a fern-leaf-shaped, grayish colony, very granular in middle, becoming almost transparent near the edges. It has no bad odor. The surrounding media has a green fluorescence. The colonies are on the surface smooth and shiny. On gelatin is similar, it does not liquify.

Agar Slant: A dull gray color, smooth along the path, many small colonies are distributed along the edge of the main growth, with a fluoresence of the underlying media of a greenish cast.

Gelatin Stab: At first there is only a small surface growth but later is developed a very scanty growth occurs along the line of inoculation, while the surface growth is an opalescentcovering. The upper part of the gelatin has a green fluorescence.

Milk: There is no coagulation nor liquifaction of the caesin.

Litimus: It has turned the litimus blue.

Fermentation tube: No gas is formed, no growth in closed arm.

Indol: Only a trace if any.

Reduction: It reduces nitrates.

Bouillon: Liquid turbid with sediment and a membraneous pellicle. Later the liquid clears and it acquires a green fluorescence.

Potatoe: A dirty white growth not very luxuriant nor spreading. It changes the color of the potatoe to a grayish blue.

Remarks: This is commonly called Bacillus fluorescence non-liquifaciens which differs from Puditus in not having that characteristic odor and from liquifaciens in that this does not liquify the gelatin. It occurs quite frequently but does not seem to be one of the active sewer bacteria.

XVII. PSEUDOMONAS PUTRIDA. (FLUGGE).

A small short rod, of which the single ones appear very much like a cocci, mostly in pairs, a few single. The width about

1-2 n and length varying from 1 1/3 - 2 times the width. It is actively motile. Observed no spores. Grows best at room temperature and is aërobic.

Plate Colonies: The surface colonies are a brownish white color, granular center spreading with very irregular edges, smooth and shiny, giving off a very bad odor on removing the cover of the dish. It gives a green fluorescence to the surrounding media. On gelatin does not liquify.

Agar Slant: A dirty white, spreading growth, abundant, edges irregular but sharply defined. Surface smooth and shiny.

Gelatin Stab: Only a slight trace of growth in the gelatin along the line of puncture. On the surface is formed ~~a dirty~~ as a dirty whity layer giving a greenish shimmer to the upper part of the gelatin only. Also has the bad odor as on agar.

Fermentation Tube: No gas, no growth in closed arm.

Bouillon: The liquid is turbid, a slight whitish sediment but no pellicle. The fluorescence here is only very slight.

Potatoe: A thin shiny brownish layer is formed, not very luxuriant.

Remarks: Commonly called Bacillus fluorescence Putidus (Flügge). I did not grow it on all the different media, for I was convinced by its fluorescence and the odor that this must be the species described by both Migula and Stemberg answering all of their descriptions. It did not occur very frequently; it occurs in almost all waters and it may not be considered as one of the active workers in this water.

XVIII. BACTERIUM PYOCINNABAREMN (Kruse).

It is a small bacillus with rounded ends length from two to four times the width, mostly single, some in chains of three and four. It grows on ordinary nutritive media, better at 37° C., but with scarcely any color formed here. It is not motile and observed no spores. An aërobic species.

Plate Colony: On agar at the end of two days they are of ochraceous rufus color with concentric rings, showing the ages in growth, edges not smooth under the microscope; it is very granular and on gelatin the irregular edges extends its projections into the surrounding gelatin. They are then swimming colonies, not coloring the media in which they are imbedded.

Agar Slant: The color is first yellowish which with age becomes a deep red. Edge smooth, surface moist, slightly granular near edges. In the incubator has hardly any color,- the deepest color is produced in the dark. Different shades are obtained with different cultures.

Gelatin Stab: At the end of several days there is a funnel shaped beginning of liquifaction, with continues, by spreading to the edges from side to side until about one half of contents is liquified. The liquid is clear with a pinkish sediment.

Milk: After several days the milk is coagulated and the caesin is liquified.

Litmus: No change in color.

Fermentation Tube: No gas is formed, growth in open bulb only.

Indol: Is not produced.

Reduction: It does not reduce nitrates.

Bouillon: The liquid is clear with a deeply pitted pellicle, at first is white which later turns pink, a pinkish sediment at bottom.
Potatoe: Growth appeared as a white growth, no apparent change in color.
Remarks: This species is "Bacillus pyocinnabareus - Kruse: this is as near as it could be place it. It agrees with Migula's description except that the potatoe growth does not turn red nor is the bouillon turbid. As this was the species that came closest to it I put it here, feeling not confident however that this is the species described by Migula. It was a very frequent occurence.

XIX. BACTERIUM LATERICEUM (Kruse).

It is a bacillus, non motile, single, and slightly curved, sometimes it grows out into filaments of several joints. The rods are about four times the width, sometimes slightly curved. No spores have been observed. It grows best at 37° C. and very slowly at room temperature; no growth from 5 - 8° C. Aërboic.
Plate Colony: It grows similar on gelatin as on agar, forming a brick red colony, small circular, sharp edged, smooth, shiny on the surface of the media. Under low power the colony is spherical, finely granular, and brownish red in color. The center is opaque with a more transparent marginal zone.
Agar Slant: The growth along the path is slightly spreading at base, at first only a flesh colored which soon turns darker. In the dark the pigment production is stronger. The edges are sharp and surface

is smooth and shiny.

Gelatin Stab: Only a slight membraneous growth along the path of needle, with a red surface growth.

Litimus Plate: Is turned blue.

Fermentation Tube: Produces no gas, with growth in open bulb only.

Indol: Is not formed.

Reduction: Of nitrates only slightly.

Bouillon: Is opalescent with a brick red sediment; no pellicle.

Potatoe: A slight growth on the surface, at first yellow and later turns red.

Remarks: This is commonly called Bacillus latericeum and is found very commonly in all waters. It has a very characteristic color which however varies with its environments. I came across it a number of times in the water taken from the different parts of the tank. There is no doubt ~~in my estimation~~ that this is the species described under this name both by Migula and Stemberg. These descriptions are however not very extensive on this species but what is given seems to apply also to this species.

XX. BACILLUS FLAVESCENS (Pohl).

It is a very short rod with square ends, non-motile or only very slight pendular motion. The rods are from $1\frac{1}{2}$ to 3 times the width. Observed no spores, grows best at room temperature. It is aerobic, non-liquifying organism producing a yellow pigment.

Plate Colony: It is when plated out from the water a large yellowish colony, the center is a yellow white; it fades to a white towards the periphery which is slightly lobed. Surface smooth and shiny. Upon making subcultures the colonies were very small about the size of a pins head, yellow and round,on the surface of the media; grow similarly on gelatin.

Agar Slant: Here it is a deep greenish yellow growth, smooth and shiny, slightly decreasing towards apex; with the middle darker than the edges; it is slightly spreading, with scarcely any growth at room temperature.

Gelatin Stab: Only a scanty growth along the path with a small yellow colony at point of inoculation. This gradually enlargens, so that after two weeks it extends from side to side; the gelatin is drying out and the surface has a cup-shaped depression. No liquifaction occured.

Milk: It remains unchanged until after two weeks when there is a yellow ring on glass and the caesin is liquified without previous coagulation.

Litimus: After four days remains unchanged.

Fermentation Tube:: No gas is formed and growth only in open bulb.

Reduction: Of nitrates does not occur.

Bouillon: The liquid remains liquified for about ten days after which it turns turbid and forms a yellow sediment, but no pellicle.

Potatoe: At first the grayish cream growth is restricted to the

line of inoculation; later it spreads and turns to a darker yellow. It changes the color of the potatoe; the growth is also slightly raised.

Remarks: This also is one of the common chromogenic species that occurs on a large number of the plates. This coincides exactly to the plus and minus characteristics with Fuller's list of water bacteria. It does not grow on the agar slant as given in Migula's description. I have found several species that will grow as separate colonies on the slant at one time and again as a united growth in other cases where subcultures were made directly from the first. The color is also not as deep as his; I called mine more a yellowish green. This may have been due to the environments.

XXI. BACTERIUM FUSCUM.(Flügge)

It is a small irregular rod varying in length; most of them are short and oval with the length 1 1/3 times the width. The width is about .5 n. The rods when in filaments can not be distinguished by the segments and are often slightly curved; the ends are rounded. It is non-motile, non-liquifying, grows best at room temperature, facultative anaërobic. Observed no spores.

Plate Colonies: The deep colonies are punctiform and yellowish brown in color. On the surface they have often an irregular, knobby form. Under the low power it is uniformly granular becoming almost transparent towards the periphery. Grows similarly on gelatin.

Agar Slant: At first it appears as a shiny, smooth, creamish growth

which gradually becomes darker especially in the middle, with the edges slightly lighter and become very ragged.

Gelatin Stab: Only a scanty growth along the path with a slight surface growth which gradually enlargens and becomes of a dark yellow or brownish color.

Milk: Remains unchanged, does not coagulate after boiling.

Litimus: Is slightly decolorized, without previous change to red.

Fermentation Tube: Forms no gas, with growth in both arms.

Indol: Is not formed.

Reduction: Of nitrates does not occur.

Bouillon: The liquid is soon very turbid, with a small ring on edge of glass and particles of growth collect which soon fall to the bottom and form a yellow sediment; no complete pellicle is formed.

Potatoe: Only a slight yellow, elevated dry growth is apparent. It is not luxuriant.

Remarks: This is commonly called Bacillus fuscus. The characteristic of its chemical behavior corresponds to Fullers species given in his table of plus and minus characteristics. It also agrees with the more incomplete descriptions of Migula and Stemberg. It is a rather common bacillus but as it is found in waters frequently it can not be considered as an active sewage bacteria.

XXII. BACILLUS CUTICULARIS. (TILS).

XXII. BACILLUS CUTICULARIS. (Tils).

It is a short bacillus, the width about .6 n. and length varying considerably from 2-4 times the width. It is mostly single, occasionally in filaments. The motility is hard to detect, and the single ones only show a slight motility. Observed no spores. It is a liquifying, facultative anaërolic species, growing but at 37° C.

Plate Colony: On gelatin the deep colonies appear as irregular, smooth edged, brownish colonies, while those on the surface have a sharp outline and are of a yellowish brown color, with a darker center. After several days the center begins to sink and the gelatin is soon liquified. The colonies, when the gelatin is all liquified, float around on the surface.

Agar Slant: A yellow growth appears in form of separate colonies, round and smooth; it is not spreading.

Gelatin Stab: After the second day the surface growth has already sunk down into the gelatin which is soon followed by a slow liquifaction in a funnel shaped manner, then on reaching the sides of the tube it works down gradually until most of the gelatin is liquified. The liquid part is turbid with heavy growth on the top and at the bottom.

Milk: A greasy yellowish band is formed on the edge of the glass. After ten days it is coagulated and caesin liquified.

Litimus: Turned blue.

Fermentation Tube: Forms no gas with growth in both arms.

Indol: Is formed after three days.

Reduction: Of nitrates does not occur.

Bouillon: A uniform turbidity of the liquid, without the formation of an apparent pellicle.

Remarks: Migula classes this as one of the sewer bacteria. It corresponds to his description with the exception that I have observed no pellicle and have found the motility very weak. It is a very common occurrence in the water examined.

XXIII. STREPTOCOCCUS CITREUS. (Eisenberg).

It is an aërolic, non-liquifying, non-motile micrococcus. The individual cells are perfectly round, rather large on an average of about 1.5 u in diameter. They are frequently separate, some in pairs and often in chains of less than 8 segments. It grows in the usual culture media at room temperature, but better in the incubator at 37°C. No growth at a temperature of 5 - 12°C. Chromogenic.

Plate Colony: It is a large lemon colored colony. Through the low power it is seen to be heavily granular in the center and almost transparent at the margin. The margin is sharp but not regular. The colonies appear on the surface of the media and are smooth and shiny.

Agar Slant: A heavy lemon colored growth along the path of the needle, spreading at the base, with irregular outlines; at first is cream colored which later becomes darker.

Gelatin:Stab: A very scanty growth along the line of puncture, with

a yellow surface growth.

Milk: Not coagulated until after boiling.

Litmus: Remains unchanged.

Fermentation Tube: Does not form gas and growth only in the open bulb.

Indol: Is not formed.

Reduction: Of nitrates only slightly.

Bouillon: Is opalescent with yellowish sediment and a ring on the edge of the glass.

Potatoe: A waxy yellow pasty growth, beaded and raised; it is limited to the path but is quite abundant.

Rosalia Acid: Is not changed.

Remarks: This species is commonly called micrococcus citreus, but called a streptococcus by Migula. The characteristic of this species as ~~I have~~ observed ~~then~~ agree very closely to those given by him. It is a very common water bacterium and is probably not one of the active sewer bacillus. It occurred very frequently in this work and was characteristic by its color.

XXIV. Micrococcus Versicolor.(Flügge)

It is a facultative aërolic non-liquifying, chromogenic micro-coccus. The organism is small and round associated in pairs and in irregular groups. It grows in the usual culture media at room temperature.

Plate Colonies: The superfical colonies on gelatin are flat and irregular in outline, smooth and have a pearly lustre, a dark yellow; while the deep colonies are more spherical in outline. Under the low power they are granular, a heavy brown in the middle and

another band at the periphery. The heavy granules are distributed irregularly.

Agar Slant: A brownish growth along the path, smooth and has a metallic lustre, with irregular edges, darker in the middle; the growth is thick and heavy.

Gelatin Stab: Small, spherical yellow colonies are developed along the line of puncture with a yellowish growth having a pearly lustre on the surface.

Milk: Is coagulated after ten days with liquifying the caesin.

Litmus: Was not changed.

Fermentation Tube: Forms gas of about 20% with growth in both arms at the end of three days.

Indol: Is formed in very small quantity.

Reduction: Of nitrates only partially.

Bouillon: Is made turbid, with a heavy brownish sediment and granules distributed through the liquid. On the surface is formed a whitish pellicle extending from side to side. It is net-work, and is not entire.

Potatoe: A clay-colored pigment is formed here, the growth is very spreading, covering almost the entire surface.

Remarks: This species is very incompletely described by Migula. This agrees with him however with the exception of the pigment as here observed it was more a brown than a greenish yellow. This description agrees very closely to Stemberg's as far as he goes. This species has not occurred very frequently.

XXV. MICROCOCCUS TARDIGRADUS. (Flügge).

A small round coccus, single, in pairs and in irregular groups. It stains well with ordinary dyes. It is non-liquifying chromogenic species. It grows in ordinary culture media at room temperature.

Plate Colony: On gelatin they are very small round colonies, the color of the gelatin, those on the surface project slightly above the media. Under the microscope they are uniformly granular, a sharp edge and have a greenish cast. The center is surrounded by a darker band; the entire colony can be removed by the point of the needle. It turns to a deep brown later. On agar the colonies become transparent at the periphery.

Agar Slant: An ochre-yellow growth, limited to the path of inoculation. It is a smooth and horny growth; spreads only slightly. It grows also in the incubator.

Gelatin Stab: The lower part of the stab is in separate colonies, near the top the stab is wider and more membraneous. There is a small yellowish growth at the point of inoculation.

Milk: Did not coagulate within twenty days.

Litimus: Is not changed.

Fermentation Tube: No gas is formed; growth in both arms.

Indol: Is not produced.

Reduction: Of nitrates does not occur.

Bouillon: After two days is turbid, with a slight white sediment at the bottom of the tubes.

Potatoe: Only a slight growth limited to the path of inoculation. Creamish and slightly raised.

Rosalinic Acid: Is not changed.

Remarks: This is micrococcus flavus tardigradus according to Stemberg and is commonly found in all waters. It has occurred frequently in this work, with its source probably from fresh waters.

XXVI. MICROCOCCUS FOETIDUS. (Liborius).

It is an aërobic, liquifying non-chromogenic micrococcus. The cocci occur single and some in chains of different lengths; many are in pairs.

Plate Colonies: On the agar to the naked eye it is a porcelain white round, shiny, smooth colony, without any kind of surface markings. It is on the surface of the media and projects slightly above the surface; through the microscope it is granular decreasing towards the periphery where it is almost transparent. On gelatin the colonies begin to sink after six days. The colonies have a dark center here and begin to liquify the gelatin after a week.

Agar Slant: It grows very slowly but a little better at the room temperature than in the incubator. It is a waxy growth, smooth and shiny; spreading at the base.

Gelatin Stab: After one day there is a slight surface growth which gradually increases until after four days when the colony begins to sink in the gelatin. A membraneous sack is formed half way down the line of inoculation at the end of which heavy lumps of yellowish growth are deposited. This sack now changed into a funnel

increases extending from side to side and gradually downward. The liquid is very turbid.

Milk: Is coagulated within ten days. The caesin is partly liquified and it has a pinkish tinge.

Litimus: Remains unchanged.

Fermentation Tube: No gas, no growth in the closed arm.

Indol: Is formed in a very slight quantity.

Reduction: Of nitrates does not occur.

Bouillon: Liquid turbid, no pellicle, with heavy white sediment.

Potatoe: A limited, beaded, raised growth, grayish in color along the line of inoculation.

Remarks: This agrees very closely to the descriptions of this species as given both by Migula and Stemberg. In this work it has not appeared very frequently.

XXVII. MICROCOCCUS CINNABARINUS.(Zimmerman).

It is a small coccus, some single, others in pairs and some in irregular masses. Frequently the division between the cells can not be seen. It is non-motile, non-liquifying, chromogenic, and observed no spores. Aerboic.

Plate Colony: The colony when taken from the plate containing the colonies of the water, it is a deep flesh colored, smooth, round, shiny and quite small soft colony. Under low power it is uniformly granular, darker in the center and becoming almost transparent towards the periphery. After several subcultures the colonies

become darker as they also will with age.

Agar Slant: At first a very faint creamish tint, which gradually becomes darker until it has a brownish red color. The growth is very soft, glistening, smooth surfaced and seems to have a granular contents; spreads very slightly near the base of the tube. It grows very slowly but better at room than at higher temperature.

Gelatin Stab: Growth extends only part of the way down the line. Here are small white, round colonies scattered along with a slight surface growth. The growth sank into the media with the gelatin evaporated, probably due to the age of the gelatin. There was no other sign of liquifaction here or on the plate.

Milk: Is not coagulated within twenty days.

Litmus: Is decolorized from the botton upwards, with a pinkish sediment and a white pellicle.

Fermentation Tube: No gas is formed and growth in open bulb only.

Indol: Is not formed.

Reduction of Nitrates: Does not occur.

Bouillon: Is opalescent with pinkish sediment, observed no pellicle.

Potatoe: Succeeded in getting only a very slight growth along the path of inoculation.

Remarks: This seems to answer the description given by Migula P.164. It occurred very frequently. It appears however as a flesh colored colony which later becomes a little darker, but the pigment is not as well developed as it is after several transplantations.

XXVIII. BACILLUS VULGARIS. (Hauser). Mig.

It is a small bacillus with rounded ends, the width about .6 μ and varying in length from 2 - 5 times the width. It is actively motile, aerobic and facultative anaerobic; observed no spores. They are single in pairs and some in short filaments which are frequently bent and twisted. It grows well on the usual culture media, at room temperature and also at 37°C.

Plate Colony: The colonies at first appear as grayish spots which rise to the surface and spread out, now having a smooth and shiny surface. The center is somewhat elevated. Through the microscope with slight magnification it has lobed edges which sometimes send out buds of new growth, but are sharply defined. The periphery is very finely grained while at the center and around it there are heavy dark clusters of granules. There is a slight radiation extending from the center outwards. On gelatin they sank to the bottom but it did not liquify the gelatin.

Agar Slant: A uniform bluish gray shiny growth spreads over the surface within twenty-four hours.

Gelatin Stab: There is growth all along the path with surface growth gradually spreading to the sides of the glass; along the path it is beaded, later turning brownish and a membrane is formed around it, but I have not succeeded in getting it to liquify.

Milk: Is coagulated; no liquifaction of caesin.

Litmus: Turns red.

Fermentation Tube: Gas is formed, about 45% with growth in closed and open arms.

Indol: Is not formed.

Reduction:of Nitrates: Does not occur.

Bouillon: Soon becomes very turbid, there is a ring on the glass and upon shaking there are clouds of particles floating which soon settle at the bottom.

Potatoe: A slightly spreading,greenish dirty growth, slightly granular changing the color of the potatoe to a bluish gray.

Remarks: I ran this through with a stock culture from Novy's and found that they agreed throughout. I could not make either of them to liquify the gelatin. With this exception it agrees closely to Migula's description. Also the analysis of the gas agrees with that of Theobald Smith of the same species. This was about the most abundant species found; there was at least one colony on every plate that was plated and on many, many more. It is probably one of the most active ones found.

XXIX. BACTERIUM PARVUM. (Lüderitz).

It is a short, thick, almost oval bacillus, with rounded ends. The width is about 1 u and the length being about 1 1/3 times that of the width. They are single some in pairs and some in rows of not more than four. Observed no spores; non-motile.

Plate Colony: A small grayish-white round colony, shiny and soft. On gelatin they sink and liquify the contents.

Agar Slant: It grows abundantly, in forms of small grayish-white colonies which later become confluent. It forms a soft stringymass,

with surface irredescent of blue, green and red. It has a fecal odor.

Gelatin Stab: Growth along the path in separate colonies. The surface growth spreads and liquifies the gelatin from side to side. Soon the entire contents is liquified; the liquid is quite turbid with heavy growth on surface and at the bottom. On glucose gel. gas is formed previous to liquifaction.

Milk: Coagulated.

Litimus: Turned red.

Fermentation Tube: Gas is formed about 26% in 2% glucose broth.

Reduction: Strongly reduces.

Potatoe: A white, shiny, soft growth, same color as potatoe, which soon drys up and disappears.

Bouillon: An incomplete pellicle is formed, liquid becomes turbid and a granular sediment is formed. The pellicle soon falls and leaves a ring on the edge of the glass.

Remarks: Gas was not formed every time. It closely agrees to Bacterium parvum or Bacillus liquifaciens parvus more commonly called. It is not an obligatory anaerobe, but grows well and almost better in the absence of oxygen. The colony was isolated from the water by planting or growing an inoculation from the water in a glucose broth sewer agar, where it first appeared as a small colony and gradually increased finally forming a semi-spherical irregular colony from which subcultures were made.

XXX. BACILLUS.----------.

[redacted]: It is a very short bacillus, about .6 u wide and 1.2 u. long. They are frequently in filaments where the separate segments cannot be distinguished; frequently chains of 5 u - 8.5 u are seen. It is slightly motile; it was difficult to determine if any regular motility or only the browning motion. It is not liquifying, non-chromogenic, facultative, anäerobic. Observed no spores; it grows in the ordinary culture media at room temperature and also at 37°C.

Plate Colony: To the naked eye it is a white, glistening circular colony at the surface and small oval and circular denser ones at the bottom. Through the microscope its contents appear as a yellow granular mass, denser at the center; at the edge it is finely grained and becomes transparent at the periphery. The colonies on gelatin are almost transparent, slightly spreading not reaching over a diameter of 1.6 in.

Agar Slant: A porcelain white, smooth growth near apex and edges; it is in form of small individual colonies. It is slightly spreading at base.

Gelatin Stab: A uniform growth along the path. A gas bubble soon opens near the surface and the growth spreads and covers its walls.

Milk: Is coagulated.

Litimus: Is turned red.

Fermentation Tube: Gas is formed about 60%; growth in both arms.

Indol: Is formed in sugar free broth.

Reduction of Nitrates: Does not occur.

Bouillon: Is soon turned turbid, with a heavy sediment and a ring on the edge of the glass; no pellicle was apparent. It turns slightly pinkish after a week.

Potatoe: A luxuriant growth on potatoes, at first creamish in color which soon turns to a dark brown. It is smooth spreading and slightly raised. It changes the color of the potatoe to a bluish gray.

Remarks: There was found no species to which this confirms but it is very closely related to B. Coli or B. aerogenes. It differs from the first in not forming any pellicle and not reducing, in chemical reaction, and from the latter in not forming any pellicle, being slightly motile and in not having the characteristic growth of the gelatin colony.

XXXI. BACTERIUM.

It is a small bacillus, usually in pairs, non-motile, liquifying, aerobic. It grows in ordinary culture media at room temperature. Observed no spores.

Plate Colony: On agar it is a whitish cream colony, those on surface are smooth and shiny, while those imbedded are denser. Under low power it appears as a spongy mass, brown color and very granular in the center. Later it becomes a dark ochre color with surface colonies glistening.

********** Agar Agar: At first a yellowish shiny growth, decreasing towards the top with lateral hair-like projections which also

decrease towards the top. Later it becomes very fluffy, irregularly branches; deep brown color. The underlying agar also takes that color.

Gelatin Stab: At first a uniform growth along the path, a bubble is soon formed with a membraneous sack surrounding it; this enlargens and liquifies the gelatin in a funnel shaped manner, then reaching from side to side and working down to the end of the stab. The liquid part is turbid with sediment at the botton of sack.

Milk: Is coagulated and caesin is also partly liquified.

Litimus: Remains unchanged.

Fermentation Tube: No gas and no growth in the closed arm.

Indol: None:

Reduction of Nitrates: Does not occur.

Bouillon: Turbid, only a slight indication of pellicle with heavy yellow sediment.

Potatoe: A dull, moist, spreading grayish-brown growth. It turns the color of the potatoe to a grayish blue, later it spreads over the entire surface, becomes slightly wrinkled and gray.

Remarks: ~~xxxxxxx~~ Nothing was found which corresponds to this in either Migula or Stemberg, but it does agree with all the characteristics of B. desidiosus given by Fuller in his table on the bacteria found in the Ohio River. This species is not given by Migula.

XXXII. BACILLUS. ----------.

It is a very short, small bacillus, its length is about 1 1/3 times the width. They are single, some in pairs and a few in filaments of more than two. It stains well with anelyne dyes. Observed no spores, motile, non-liquifying, non-chromogenic, aerobic. It grows well in ordinary culture media, better at room temperature than at 37°C.

Plate Colony: It is a very thin, circular almost transparent, flat and lying on the surface of the media. It is slightly irridescent. Through the low power it is coarsely grained in the center and becomes more uniform towards the edge. On gelatin it is a round creamish colony with a yellow center and a sharp smooth edge. Under low power the periphery has a greenish cast and granular bundles radiate from center to the periphery.

Agar Slant: The growth here is in form of small round colonies, distributed over the entire surface, some of which later become confluent.

Gelatin Stab: Growth along the path only near the point of inoculation, with a slight surface growth. It does not liquify the gelatin.

Milk: Not coagulated. It had a very bad odor.

Litimus: Unchanged.

Fermentation Tube: No gas, growth in open bulb only.

Indol: None.

Reduction of Nitrates: Does not occur.

Bouillon: Liquid turbid, heavy white sediment, a pellicle of lace-like strips extending from one edge of the glass to the other, later

they become confluent and almost completely cover the surface.
Potatoe: A dirty yellow, smooth, shiny, restricted to path but quite luxuriant growth, which changed the color of the potatoe to a grayish-brown.
Remarks: I have not succeeded in finding any species described by the different authors to which this description would apply. It was not very commonly found nor did it seem to be a very prominent one when it did occur.

XXXIII. BACILLUS.

It is an oval bacillus which can hardly be distinguished from a coccus. It is slightly motile at times, and quite so at other times. Most of them occur single, while some are in pairs. It is non-liquifying, non-chromogenic, facultative anaerobic. Observed no spores. It grows well at both room temperature and at 37°C.
Plate Colony: It is a large white, shiny, smooth surfaced colony, having concentric rings. The middle is slightly irridescent and has a metallic lustre. The edges are lobed and heavier than the middle part. Through the low power the margin is granular then this is followed by a more transparent layer, which is bordering a peculiarly notched heavier band(which may only have been the margin) and then the lighter middle part. On gelatin the colonies are yellowish small and round with a darker center.

Agar Slant: A porcelain white, soft, smooth growth with irregular edge and slightly spreading at the base.

Gelatin Stab: Along the path is a uniform granular, creamish growth with slight surface growth. It does not liquify the gelatin.

Milk: Coagulated.

Litimus: Turns red.

Fermentation Tube: Gas, about 52%. Growth in both arms. (See gas analysis later).

Indol: Faint test.

Reduction: Strongly reduces.

Bouillon: Liquid very tinted, pellicle brittle and granular which upon slight shaking breaks and falls which soon deposits at the bottom of the tube.

Potatoe: A grayish, rough, irregular slightly spreading growth. It has changed the color of the potatoe to a bluish gray.

Remarks: It acts very much like B. album, but the gas analysis show that it is quite a different species. It also differs from it in forming some indol. According to the gas analysis it would agree very closely with B. ubiquitus yet the colonies did not appear alike and this species is motile.

XXXIX. PLANOCOCCUS.---------- --.

It is a small diplo-coccus, motile; observed no spores. It is non-chromogenic, non-liquifying, aërobic, grows on ordinary culture media, from 10° - 37° C. but best at room temperature.

Plate Colony: On agar a very small almost transparent colony. On gelatin it is perfectly circular with sharp edges, uniform, bright and shiny, has a yellow mixed with green tinge. They appear on the surface of the media.

Agar Slant: A porcelain white growth in form of separate colonies which later become confluent. It has a faint irridescence with reflected light.

Gelatin Stab: At first a uniformly granular growth along the path. A gas bubble breaks the upper part of the gelatin and growth spreads along the edge. After several weeks it was liquified.

Milk: It has a bad odor and coagulated after boiling.

Litimus: Not changed.

Fermentation Tube: No gas, no growth in closed arm, with heavy growth in open bulb.

Indol: None.

Reduction: No reduction of nitrates.

Bouillon: Liquid uniformly turbid; no pellicle; a white ring on the edge of the glass, a dirty white sediment. Liquid later turns ruddy.

Potatoe: A heavy beaded limited creamish growth which later becomes yellow in color and spreads over the entire surface and is slightly wrinkled.

Remarks: It does not seem to be a very prominent worker and as far as I could tell it did not appear very frequently. ~~I have found~~ No corresponding species in Migula was found.

XXXV. MICROCOCCUS.----------.

It is a small round coccus which occurs single and in pairs. When in pairs it is slightly motile. It does not stain very readily with anelyne dyes. It is non-liquifying, non-chromogenic, facultative anäerobic, and grows well on ordinary culture media at room temperature but better at 37°C.

Plate Colony: The original colony from which the subcultures were made was a triangular dense mass which was roughened by small colonies from all sides. The subcultures resulted in oval, triangular and circular colonies in the depth and those on the surface were larger round, yellowish, finely granular and fading towards the periphery.

Agar Slant: A heavy, whitish-gray smooth thin growth along the whole length, edges lobed. In the middle there is an elevated line and also at the edge.

Gelatin Stab: A very thin granular growth along the line which is roughened by projecting colonies. There is a slight growth on the surface. It does not liquify the gelatin.

Milk: Coagulated.

Litmus: Turned red.

Fermentation Tube: Gas is formed; growth in both the closed and open arms.

Indol: A slight trace.

Reduction: Nitrates are reduced only slightly.

Bouillon: Liquid becomes turbid, a white sediment and a white granular incomplete pellicle.

Potatoe: A dirty creamish, limited, raised, moist growth. It changes the color of the potatoe, and gas bubbles arise around it.

Remarks: This is another one of the bright, shiny, soft colony which seem to be characteristic to the sewer bacteria.

XXXVI. BACILLUS.----------.

It is a very short, oval bacillus, motile, single and some in pairs. Observed no spores and is chromogenic, liquifying and facultative anaerobic. It is difficult to stain.

Plate: It is a soft shiny colony slightly projecting above the surface; the central part is yellow while the surrounding outer part is almost white. Through the microscope the periphery is almost transparent, while towards the middle it is heavily granulated.

Agar Slant: A soft, smooth growth slightly spreading at base, and yellowish in color. When grown at room temperature it has a deeper color, but it grows better at 37°C.

Gelatin Stab: There is first only a membraneous growth along the path with an irregular surface growth, which gradually sinks and begins to liquify from side to side, and continues to work downward until about one half of the contents is liquified. The liquid part is filled with floating floculent particles.

Milk: Not coagulated.

Litimus: Slightly pink and almost entirely decolorized.

Fermentation Tube: No gas; growth in both arms.

Indol: None.

Reduction: It does not reduce.

Bouillon: Liquid turbid, slight ring on glass, slight white sediment and no pellicle.

Potatoe: A soft yellow growth limited to the point of inoculation.

Remarks: This is one of the less common species, it having occurred only a very few times.

XXXVII. BACILLUS.----------.

It is a very small bacillus about 8 μ wide and about from $1\frac{1}{2}$ - 3 times as long; ends rounded; single and frequently in pairs. Observed no spores, and is non-chromogenic, non-liquifying, facultative anäerobic. It grows on ordinnary culture media at room temperature but better at 37°C.

Plate Colony: It is a thin white, flat colony, edges lobed. Heavily grained near the center and has a slignt greenish cast.

Agar Slant: At first a beaded growth along the path, white, later become confluent giving it a soft white smeary appearance. It has a pearly lustre.

Gelatin Stab: A uniform rough heavy growth along the entire path, and a surface growth which spreads from side to side.

Milk: Coagulated.

Litimus: Red.

Fermentation Tube: No gas; growth in both arms.

Indol: none formed.

Reduction: No reduction of nitrates.

Bouillon: Liquid becomes turbid with a heavy sediment and particles distributed throughout the liquid which settles on the sides of the tube.

Potatoe: A light brownish color, spreading, granular, which later turns to a pinkish brown and gives off a very disagreeable odor.

Remarks:

XXXVIII. BACILLUS. ----------.

It is a very short oval bacillus, single, in irregular groups and a few in short chains; width equals to about .5 u and 1 1/3 - 2 times as long. In broth the rods are longer than on agar. Observed no spores; is motile, liquifying, non-chromogenic, aerobic. It grows on ordinary culture media, best at room temperature.

Plate Colony: It is at first a small bluish white colony, about the size of a pin's head in the depth of the media, it soon comes to the surface and begins to spread and forms a greenish yellow branched colony. Under the low power it is marked with numerous threads.

Agar Slant: Growth all along the path of the needle, more near the base where it is spreading and is granular. The edges are sharp

and smooth.

Gelatin Stab: At first only a slight growth along the path and at the point of inoculation; soon a membraneous growth is found around the growth along the path. The surface growth then sinks and forms a cup-shaped appearance, it continues to sink farther and farther so slow that the cup contents is evaporated. The membrane then takes on a beet shaped appearance and works from side to side and liquifies the gelatin. The liquid part is clear, with surface growth and sediment.

Milk: Not coagulated.

Litimus: Remains unchanged.

Fermentation Tube: No gas; no growth in closed arm.

Indol: None.

Reduction: It reduces nitrates.

Bouillon: Liquid slightly turbid, no pellicle, a slight yellowish sediment.

Potatoe: A lemon yellow, thin smeary growth, slightly spreading and changing the color of the potatoe to a bluish gray.

Remarks:

XXXIX. MICROCOCCUS. ----------.

It is a small micro-coccus, usually in pairs, frequently inirregular groups; they measure about 8 u in diameter; not motile. Observed no spores. It grows well at room temperature but better

at 37°C.

Plate Colony: A very small, round, shiny, smooth, greenish colony on the surface of the media. Under low power it is a greenish yellow granular mass becoming more uniform towards the periphery; after a short time the colony spreads slightly and forms an irregular but sharply defined edge.

Agar Slant: A whitish, slightly raised smooth growth, slightly spreading at base with irregularly notched edges.

Gelatin Stab: First only a slight growth along the path with a slight surface growth which gradually increases and soon is depressed in the media and liquifaction commences from side to side and downwards one half inch. The liquid part is turbid with heavy growth at the bottom. Gradually the entire contents is liquified.

Milk: Coagulated after boiling.

Litimus: Decolorized.

Fermentation Tube: No gas; no growth in the closed arm.

Indol: None.

Reduction: No reduction of nitrates.

Bouillon: Turbid, white sediment.

Potatoe: A greenish yellow, raised irregular growth; quite luxuriant.

Remarks: They stain very readily, also by Gram's method.

XL. BACTERIUM.----------.

It is a slender bacillus, the width being about .8 u and the length varies from 3 to 5 times that of the width; the ends are rounded; spores not observed; not motile.

Plate Colony: It is an orange colored, irregular colony on the surface of the media, under low power there is seen in the center a dark dense center surrounded by heavy granules which gradually fade toward the ragged periphery.

Agar Slant: At first a grayish white, shiny, smooth growth along the line of inoculation which later turns to a pale yellow with notched and senate edge, decreasing towards the top. It does not grow at 37°C.

Gelatin Stab: Only a slight growth along the upper part of the stab with a surface growth which soon extends from side to side.

Milk: Not coagulated.

Litimus: Is gradually decolorized from the bottom up.

Fermentation Tube: No gas formed; no growth in the closed arm.

Indol: None produced.

Reduction: Reduces slightly.

Bouillon: Liquid turbid, with slight white sediment, a white ring on the edge of the glass with a stringy growth hanging down from this.

Remarks: It has occurred quite frequently in this work more at times than others.

XLI. BACILLUS.----------.

It is a slender bacillus, width about .6 u and varies in length from 3 to 4 times the width. They are single and in pairs; actively motile, and observed no spores.

Plate Colony: A round, sharp-edged colony, almost transparent. Under low power it appears like a mass of granules with no back ground. The deeper colonies are smaller and denser.

Agar Slant: A very thin growth, spreading at base and decreasing towards the apex; grows better at 37°C.

Gelatin Stab: Growth all along the path in form of separate colonies closely connected but are separate near the tip. A small grayish white surface growth.

Milk: Coagulated; liquified the caesin and forms a pellicle on the surface.

Litimus: Turned red.

Fermentation Tube: Gas is formed, about 27%. Growth in both arms.

Indol: A small amount of indol is formed.

Reduction: Nitrates are reduced.

Bouillon: Liquid very turbid with suspended particle distributed; on the surface a pellicle is formed which has a greasy appearance, is thin and irridescent.

Potatoe: A yellowish, shiny, elevated irregular growth, slightly beaded near the tip while the rest is spreading.

Remarks: It has a very bad, fecal odor. Stabs of this were made in plain gelatin, sewer-broth gelatin and sewer gelatin; the growth in the three was similar but more abundant in the latter two; the

growth was very heavy all along the path, while in the former there was a heavier surface growth. In the sewer and sewer-broth gelatins gas bubbles are formed, in all cases the bubbles were not in contact with the stab which shows that the organism forms a soluble ferment.

XLII. BACILLUS.----------.

It is a small bacillus with rounded ends; width about .6 μ and the length varying from 1½ to 3 times the width. It occurs single and some in long chains of 4 - 12 organisms. The segments can be easily distinguished. No spores were observed. The bacillus is actively motile; both the single and long filaments.

Plate Colony: A white glistening round colony, sharp edges.

On gelatin the surface colonies sink at the end of the third day. Under low magnification they appear as a gray uniformly granular mass.

Agar Slant: A rough, grayish-white growth along the path, decreasing towards the apex; edges very irregular, which later developed into a smooth, shiny, sharp edged growth.

Gelatin Stab: The growth is at the surface and spreads from side to side and sinks into the gelatin, thus liquifying the upper half of the contents. The liquid is very turbid and has a heavy yellowish sediment.

Milk: Coagulated.

Litimus: First red then blue.

Fermentation Tubes: No gas; slight growth in closed arm.

Reduction: Reduces only slightly.

Bouillon: Liquid turbid, with white suspended particles; a thin membraneous pellicle which falls to the bottom on slight shaking. The liquid later becomes opalescent and contains a heavy sediment.

Potatoe: At first only a very slight growth, the color of the potatoe which later becomes a smeary, brownish, raised growth, which spreads over almost the entire surface.

Remarks: This did not liquify every time, when not, it formed a simple growth along the path with a slight surface growth.

Name of organism.	number	acid	filaments	motile	spores	Broth pellicle	Broth turbid	agar stabs dull	agar stabs wrinkled	agar plate spreading	Potatoe visible	Potatoe luxuriant	Growth in cloud arm	aërobic	temp. in C.	liquefaction Gelatin	liquefaction Casein	gas formed	nitrates reduced	curd formed	milk coagulated	fecal odor	chromogenic	fluorescent.
Bacillus subtilis	I	−	+	+	+	+	−	+	+	+	+	+	−	+	37	+	+	+	+		+	+	−	−
Bacterium ubiquitum	II	+	+	−	−	+	+	−	−	−	+	+	+	+	22	−	−	+	+	+	−	+	−	−
Bacillus coli-communis	III	+	−	+	−	+	+	−	−	−	+	−	+	+	37	−	−	+	+	+	−	−	−	−
Bacterium canalis	IV	+	−	−	−	−	+	−	−	−	+	+	+	+		+	−	+	−	+	+	−	−	+
Bacillus myxoides	V	−	+	+	+	+	−	+	+	+	+	+	+	+	37	+	+	+	+	+	+	−	−	+
Bacillus subviscosus	VI	−	−	−	−	−	−	−	−	−	−	−	−	−	22	−	−	−	−	−	−	−	−	−
Bacillus viscosus	VII	+	+	+	−	+	+	−	−	−	+	+	−	+	22	−	+	+	+	+	+	−	−	−
Bacterium pyogenes	VIII	+	+	+	−	+	+	−	−	−	+	+	−	+	22	−	−	+	+	+	+	−	−	−
Bacillus nebulosus	IX	−	+	+	−	+	+	−	−	−	−	−	−	+	37	+	+	+	+	+	+	−	−	−
Bacterium aërogenes	X	+	−	−	−	+	+	−	−	−	+	+	+	+	37	−	−	−	+	+	+	−	−	−
Bacillus ramosus	XI	−	+	+	+	+	−	+	+	+			−	+	22	+	+	+	+		+	+	−	−
Bacterium album	XII	+	−	−	−	−	+	−	−	−	+	+	+	+	22	−	−	+	+	+	+	−	−	−
Bacillus hyalinus	XIII	−	+	+	−	+	+	+	+	+	+	−	−	+	37	+	+	+	+	+	+	−	−	−
Bacillus	XIV		+	+	−	+	+	−	−	+	+	+	−	+	37	+	+	+	+	+	+	−	−	−
Pseudomonas fluorescens	XV	−	−	+	−	+	+	−	−	−	+	+	−	+	22	+	+	−	+		+	−	−	+

Name of organism	number	acid	filaments	motile	spores	pellicle	broth turbid	agar slabs dull	agar slabs wrinkled	agar plate spreading	potato visible	potato luxuriant	Grown in closed arm	aerobic	temperature in C.	gelatin liquefied	casein	gas formed	nitrates reduced	indol formed	milk coagulated	Fecal odor	chromogenic	fluorescent
Pseudomonas Paraiinii	XVI	−	−	−	−	+	+	−	−	−	+	−	−	+	22	−	−	−	+			−	+	+
Pseudomonas putrida	XVII		−	+	−	−	+	−	−	+	+	−	−	+	22	−		−				+	−	+
Bacterium hyacinthinum	XVIII	−	+	−	−	+	−	−	−	−	+	−	−	+	37	+	+	−	−	−	+	−	−	−
Bacterium laterieum	XIX	−	−	−	−	−	+	−	−	−	+	−	−	+	37	−	−	−	+	+	−	+	+	+
Bacillus flavescens	XX	−	−	+	−	−	+	−	−	−	+	−	−	+	22	−	+	−	−	−	−	−	+	+
Bacterium fuscum	XXI	−	+	−	−	−	+	−	−	−	+	−	+	+	22	−	−	−	−	−	−	−	−	−
Bacillus cuticularis	XXII	−	−	+	−	−	+	−	−	−	−	−	+	+	37	+	−	−	−	−	+			
Micrococcus citreus	XXIII	−	+	−	−	−	+	−	−	−	+	+		+	37	−	−	−	+	+	−	+	−	+
micrococcus variabilis	XXIV	−	−	−	−	+	+	−	−	−	+	+	−	+	22	−		+	+	+	+	−	−	−
micrococcus tardigradus	XXV	−	−	−	−	−	+	−	−	−	+	−	+	−	22	−	−	+	+	−	−	+	+	+
micrococcus luteus	XXVI	−	+	−	−	−	+	−	−	−	+	+	+	+	22	+	+	−	+	+	+	−	−	−
micrococcus minimus	XXVII	−	−	−	−	−	+	−	−	−	+	+	−	+	22	−		−	−	−	−			
Bacillus vulgaris	XXVIII	+	+	+	−	−	+	−	−	−	+	+	−	+	22	−	−	+	+	+	+	+	+	+
Bacterium carneum	XXIX	−	−	−	−	−	+	−	−	−	+	+	+	+	22	+	−	+	+	−	+	+	−	−
Bacillus	XXX	+	+	−	−	−	+	−	−	−	−	+	+	+	37	−		+		−	+			
Bacterium	XXXI	−	−	−	−	+	+	−	−	−	+	+	+	+	22	+	+	−	+	−	+	−	+	−

Name of organism	Number	Acid in milk	Milk peptonized	Gelatin stab	Gelatin plate	Agar tube streak	Agar tube stab	Bouillon turbid	Bouillon pellicle	Litmus reduced anaerobically	Litmus reduced aerobically	Litmus reduced in presence of sugar	Litmus reduced in presence of peptone	Indol	Nitrites	Gas from glucose	Gas from lactose	Gas from saccharose	Gas from mannite	Gas from dulcite	Gas from glycerine	Gas from inulin	Gas from salicin
Bacillus	XXXII	−	+	+	−	+	+	−	−	−	+	+	−	+	37	−	−	−	−	−	−	+	−	−
Bacillus	XXXIII	+	−	+	−	+	+	−	−	−	−	+	+	+	37	−	−	+	+	+	+		−	−
Bacterium	XXXIV	−	−	−	−	−	+	−	−	−	+	+	−	+	22	+	−	−	−	−	+	+	−	−
Bacterium	XXXV	+	−	−		+	+				+		+	+	37	−	−	+	+	+	+		−	−
Bacillus	XXXVI	+	−	+	−	−	+	−	−	−	+		+	+	37	+	−	−	−	−	−	−	+	−
Bacterium	XXXVII	+	−	−	−			−	−	−	+	+	+	+	37	−	−	−	−	−	+	+	−	−
Bacillus	XXXVII	−	+	+	−	−	+	−	−	−	+	−		+	22	+	−	−	+	−	−	−	−	−
Bacterium	XXXIX	−	−	−	−	−	+	−	−	−	+	+	−	+	37	+	−	−	−	−	+	−	−	−
Bacterium	XL	−	−	−	−	−	+	−	−	−			−	+	22	−	−	−	+	−	−	−	−	−
Bacillus	XLI	+	−	−	−	+	+	−	−	−	+	+	+	+	37	−		1	1	+	−	−	−	−
Bacillus	XLII	+	+	+	−	+	+	−	−	−	+	+	+	+	22	+	+	−	+	−	+	−	−	−

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As has been stated before some species of bacteria have power to produce an enzyme which can break up carbohydrates into gases, Dr. Theobald Smith in the account of his classic experiments has given a number of species which produce gases and the necessary conditions under which the gases are produced, together with the composition of the same. Various analyses are on record also of the gases liberated, these analyses being more or less dependent upon the point of view of the investigator and the time when the work was done. Dr. Smith was content with the determination of the amount of carbon dioxide in the gas collected in the fermentation tube, devised by himself, by using potassium hydroxide as the absorbing material. The residual gas being found to be explosive was considered as hydrogen and calculated by difference. Pammel and Bennett have studied the action of several gas producing organisms with special attention to their behavior at different temperatures upon sugars, namely: glucose, cane-sugar, and lactose. In their work we find no mention of nitrogen, methane or any of the heavier gases, the results only of carbon dioxide and hydrogen are given. In the case of B. coli-communis at 28°C. they found 25.16% carbon dioxide and 74.8% of hydrogen.

A more extensive study upon the gas produced by B. coli-communis has been made by Mary Pennington and Küsel. They studied the influence of the time of action and the age of the organisms when acting in a glucose medium containing: beef extract ***% 5%; salt 5%; peptone 1%; glucose 1.5% and made neutral to phenolpthaleim. The results

obtained at the end of a period of 48 hours were: carbon dioxide, 34%; hydrogen, 63%; methane, 1.5%; and nitrogen, 1.5% while at the end of 192 hours: carbon dioxide, 23.59%; hydrogen 69.88%; methane, 2.39%; and nitrogen, 4.95%. The result of the analyses of the gases produced by bacteria have as yet not been very accurate; the duplicates in many cases differing by several per cents even when the greatest care has been taken to have all the conditions as near alike as possible.

It has been found that bacteria are more active in glucose medium than in any other sugar solution. The medium used in the following investigation was two per cent. glucose-broth, making this medium as nearly alike every time as possible. In this work a special study was made of the gases formed by some of the most prominent gas-producers found. The apparatus used for this purpose was two glass bulbs connected by a rubber tubing fitting tightly; the one bulb was provided with a stop cock while the other was an open bulb. These were first sterilized in an autoclave for 30 minutes, then the desired culture medium was admitted through the open bulb, the stop cock being open. The level was adjusted until the bore of the stop cock was just filled. The cock was then turned the open bulb plugged with cotton and after resterilization for ten minutes in the autoclave rendered the apparatus fit for inoculation after being cooled to about 37° centigrade. Usually four or more tests were made of each species studied, two of which were inoculated in the closed end and two in the open bulb. The inoculat-

ion was performed by slipping the platinum wire charged with the desired species, through the stop cock until it met the culture medium. For this purpose the open bulb had to be raised so that the fluid in it was above the level of the stop cock. Inoculation of the open bulbs was done by simply removing the plug and using the plain wire. It was found that at 37° centigrade there was a more rapid development of the gases than at the room temperature; hence in every case the bulbs were kept in the incubator from the time of inoculation until two hours previous to the analysis.

As the organism multiplies, the gas is liberated and is at once collected in the closed bulb, when this has been inoculated in the closed bulb, displacing the liquid and forcing it over to the open bulb. In the other case, the gas is first formed in the open bulb and consequently escapes hence no gas is collected until the organism had commenced to work on the material contained in the closed bulb.

In most cases the formation of the gases seemed to cease by the end of 45 hours, so development for that length of time was permitted before analysis. The bulbs were taken into a small cold room of almost constant temperature where all the analyses were made. For this purpose Hempel's burette was used. The carbonic acid gas (C.O.2) present in the gas was absorbed by potassium hydroxide; the oxygen (O) by pyrogallic acid, the ethane (C_2H_6) by fuming sulphuric acid, the carbon monoxide (CO) by an ammonical solution of cuprous chloride. The hydrogen (H) was tested by combustion; after the gas had been

mixed with a certain quantity of air the hydrogen was calculated as two-thirds of the contraction after combustion; the mash gas (CH_4) was calculated by the carbon-dioxide formed by the same combustion. The nitrogen (N) was determined by difference.

Irregularities arise in gas production when there is some carbohydrate contained in the meat or beef extract. A small quantity would make a difference in the amount of gas formed, and the acidity of the medium would cause a difference, as will be shown. The manipulation of the burette must be very accurate for a difference in the readings of one tenth cubic centimeter makes a difference of about .17 to .5 per cent. in the final results. Table V gives the amounts of the different gases analyzed. The composition of the gas, which is given off by the tank, as made by Dr.A.W.Palmer is as follows: carbonic acid gas (CO_2), 10.7% of total volume; free nitrogen (N_2) 27.8; marsh gas (CH_4), 55.3; ethane (C_2H_6), 6.2; no trace of H_2S was found. In this all the explosive gas was calculated as marsh gas, while in the following analysis this was separated into hydrogen and marsh gas.

Table V. Gas Analyses.

Bacterium aërogenes(escherich) X.

Time from innoculation to analysis 45 hrs.

Total Liquid	Total Gas	Place of Innoculation.	CO_2	Hydrogen	CH_4	Nitrogen.
	47	open	24.49	70.61	0	4.90
			24.33	70.59	0	5.08
270	62	open	22.91	72.29	0	4.80
			22.94	72.39	0	4.67
275	64	closed	22.11			
			22.36	72.07	0	4.57
		Bacterium album.(Mig.) XII.				
175	125	open	55.43	39.88	.776	3.914
145	84	closed	55.78	40.05	.686	3.481
			55.67	40.46	.656	3.216
150	85.2	closed	53.46	41.92	.651	3.969
			54.63	41.92	.731	2.699
		Bacillus coli-communis. (Escherich) III.				
260	40	open	27.0[illegible]	7[illegible].38		2.37
				69.10	0	3.65
2[illegible]	50	closed	25.10	70.18	1.9	2.82
				69.81	1.6	3.49
200	62	closed	28.17	69.13	0	2.70
				68.68	0	3.15
14[illegible]	56	open	28.84	65.22	0	5.94
				65.05	0	6.11

Bacterium Ubiquitum(Jordan) II.

Total liquid.	Total gas.	Place of innoculation.	CO_2	Hydrogen	CH_4	Nitrogen.
	71	open	48.73	46.53	.27	3.91
				45.23	.61	4.87
	52.1	open	48.94	46.24	.686	3.559
				45.16	.909	4.416
220	94.6	closed	53.44	39.76	0	5.374
				40.13	1.94	2.964
			Bacillus vulgaris (Hauser) Mig. XXVIII.			
270	72	open	22.22	73.12	.664	4.006
			22.04	73.52	.702	4.23
210	57 ~~13~~	open	21.23			
			21.22	74.07	.703	4.007
235	69	closed	22.66	71.70	1.40	4.20
			22.48	73.30	.73	3.49
		closed	19.56	74.77	1.13	4.54
			19.53	75.09	1.14	4.24
			Bacillus ----------. XXXIII.			
,190	74.6	open	46.78	49.71	0	3.51
				50.25	0	2.97
190	85	open	47.77	49.38	0	2.85
			47.46	49.23	0	3.31
206	146	closed	58.84	38.6	0	2.47
				37.04	0	4.12
205	100	closed	58.25	38.86	0	2.89
			58.46	---		

Bacterium canalis. (Mori). IV.

Total liquid.	Total gas.	Place of innoculation.	CO_2	Hydrogen	CH_4	Nitrogen.
190	78	open	35.71	54.6	.945	8.107
160	52	open	36.29	54.01	1.076	8.046
187	60	closed	29.95	66.18	0	3.87
				64.78	0	5.27
160	42	closed	32.21	63.48	0	4.31
				62.31	0	5.48
		Bacillus ----------. XLI.				
240	124	open	42.46	52.70	0	4.84
			40.54	55.34	0	4.12
300	120	open	42.04	54.62	0	3.34
			41.98	54.54	0	3.48
235	144	closed	48.86	48.84	0	2.30
			48.76	49.51	0	1.73
305	145	closed	47.06	48.73	0	4.21
			47.77	49.22	0	3.11

No ethane was found in any of the gases. Carbon monoxide in one case only, that was in Bacterium canalis, where one-half per cent. was found when innoculated at the open end. In Bacterium ubiquitum in addition to theanalysis given on the table one-half per cent of oxygen was found in each case.

Bacterium coniglis. (Mori). IV.

Total liquid.	Total gas.	Place of inoculation.	CO_2	Hydrogen	CH_4	Nitrogen.
190	78	open	35.71	64.6	.946	6.107
160	52	open	36.29	64.01	1.076	6.046
187	60	closed	29.95	66.18	0	3.87
				64.78	0	6.27
160	42	closed	32.21	63.48	0	4.31
				62.31	0	5.48

Bacillus ---------- XII.

240	124	open	42.46	52.70	0	4.84
			40.54	55.34	0	4.12
300	120	open	42.04	54.62	0	3.34
			41.98	54.54	0	3.48
235	144	closed	48.86	48.84	0	2.30
			48.76	49.51	0	1.73
305	146	closed	47.06	48.96	40	4.21
			47.77	49.22	0	3.11

No ethane was found in any of the gases. Carbon monoxide in one case only, that was in Bacterium coniglis, where one-half per cent. was found when inoculated at the open end. In Bacterium ubiquitum in addition to thesmalysis given on the table one-half per cent of oxygen was found in each case.

From these tables we see that Bacterium aerogenes and Bacillus coli-communis are closely related. The amount of gas formed as well as the percentage composition is very similar. Only in three cases out of the eight is there a marked change in composition between those that have been inoculated in the open and closed ends; in each of these three cases the per cent. of carbondioxide is greater in the ones that had been inoculated at the closed bulb which may be due to part of the CO_2 being dissolved as it passes from the lower end to the top.

(TABLE VI) Rate of per cent. of gas produced by Bacillus vulgaris.

(Hauser) Mig.

	Time of growth in days. 1.	2.	3.	4.	Initial acidity	Final acidity.
I	5%	30%	31%	31%	1.0	2.7
II	0	26	31	31	1.0	2.7
III	20	31	32	32	1.0	2.8

The per cent of gas produced by Bacterium album. Mig. in cultures of different ages and in glucose-broth of different acidity.

(TABLE VII). Initial Acidity .8

Time of growth.	Age of original colonies in days.				
Hours.	2.	3.	4.	5.	6.
20	25%	35%	32%	34%	29%
28	30	36	35	39	33
44	33	38	38	39	36
72	33	38	38	39	36
Final acidity	3.2	3.2	3.1	3.1	3.3

(TABLE VIII) Initial Acidity .4

Time of growth.	Age of original colonies in days.				
Hours.	2	3	4	5	6
20	45%	41%	45%	38%	40%
28	48	45	48	43	43
44	48	46	48	40	43
72	48	44	46	42	40
Final Acidity.	3.3	3.3	3.3	3.3	3.2

The three cultures, the results of which are shown in table VI, were made at the same time and under similar conditions, from a broth-culture of B. vulgaris. It shows that gases are not always formed at the same time nor at the same rate, but that the final per cent. is practically the same. This development ceases within three days. It will be noticed that the final acidity is similar in the three cases, which may be the cause of the stop of the development of the gas. The acidity as expressed above indicates the number of cubic centimeters of $\frac{N}{20}$ sodium hydroxide necessary to neutralize five cubic centimeters of the media used.

Tables VII and VIII show the relative amounts of gas formed, using bacterium album and medium of different acidity. From table VII we should conclude that the cultures of more than two days old produce more gas, while from Table VIII the reverse seems true. When the initial acidity is greater a smaller amount of gas is formed. Here again there seems to be a point of acidity after which no more

gas is formed. Where the initial acidity is lower there was a chance for 2.9 cubic centimeters of acid to be formed while in the former there seems to be a possibility of only 2.4, hence more gas in the latter. In Table VIII all the gas is formed within 44 hours and that upon standing as shown in table VIII some of the gas is again dissolved. This shows that for these two species there is a different final reaction, but that in both cases the gases are formed until their respective points are reached.

CHEMICAL ANALYSIS.

For the chemical analysis the water was collected in a five gallon keg which was well washed. The keg was filled with sewer water just as it was passing into the tank. This was done so as to get it in the fresh state before all the changes on the organic matter had commenced. The water was brought to the laboratory in the evening and kept in the cold room until the next morning when it was taken out in granite buckets and boiled. It was then well mixed and the heavy solid parts allowed to settle at the bottom. It was boiled so as to get all the soluble matter into solution. The upper liquid was decanted off in parts of 750 cc in flasks and sterilized for thirty minutes in an autoclave. Analysis of this water in duplicate was made and the sterile flasks were inoculated with pure cultures of several different species and left for seven, fourteen and twenty-one days each before analysis. The inoculations were made as follows: the different cultures were tested for purity then a new culture was made in broth, which on the following day was used for inoculating into tubes containing ten cubic centimeters of the above sterile sewage. These tubes were kept in the incubator for two days, after which they were poured one in each flask of sterile sewage. The flasks were kept at room temperature for the time stated above.

The determination of free ammonia, albumenoid ammonia, total organic nitrogen, nitrites and nitrites were made by the nesslerization method and the results calculated to parts per million. The total solids were determined by evaporating from one hundred to two hun-

dred cubic centimeters of the water.

The following are the results given in tabular form in parts per M.

Table IX.

	Original	Bacterium aërogenes			Bacterium album.		
		7 days	14 days	21 days	7 days	14 days	21 days
Free Ammonia.	14.4	15.6	16.0	15.2	18.8	12.8	16.2
	14.4	15.8	16.4	14.8	18.6	13.1	16.4
Albuminoid Ammonia.	9.0	9.5	13.0	13.0	11.7	9.7	8.85
	9.2	9.75	13.00	12.5		10.0	8.42
Total Organic Nitrogen	38.75	37.5	42.5	42.5	37.5	37.0	32.5
	39.	36.8	42.5	42.5	36.8		33.0
Nitrites	.65	.45	.125	trace	.35	.116	trace
	.66	.45	.100	trace	.35	.110	trace
Nitrates	2.23		2.195	2.4	1.73	1.894	1.44
	2.22		2.33	2.48	1.49	1.93	1.40
Total Solids per 1000	1.178	1.075	1.015	.975	.9625	.970	.896
	1.006	1.048	1.011	.940	.936		.929
Loss on Ignition.	.297	.28	.229	.255	.238	.231	.204
	.237	.28	.241	.238	.303		.206

Table X.

	Original	Bacillus coli-com unis.			B. ---------- XIV.		
		7 days	14 days	21 days	7 days	14 days	21 days
Free Ammonia	14.4	10.88	10.4		20.24	21.32	20.8
	14.4	10.72	10.8		20.8	20.8	20.4
Albuminoid-Ammonia	9.0	9.57	8.28	13.2	9.00	8.83	6.33
	9.2	9.28	8.28	14.0	7.60	8.66	6.28
Total Organic Nitrogen.	38.75	34.2	34.00	34.2	33.0	32.0	40.5
	39.0	35.0	34.50	33.5	33.5	32.5	39.5
Nitrites	.65	.233	trace	.100	.4	.20	.200
	.66	.25	trace	.083	.45	.163	.187
Nitrates	2.23	2.486	2.13	1.7	1.51	1.56	1.6
	2.22	2.583		1.76	1.44	1.71	
Total solids per 1000	1.178	.903	.994	.913	.937	.980	.996
	1.006	.900	.932	1.003	.970		
Total loss on ignition.	.297	.151	.208	.177	.204	.213	.272
	.237	.167	.195	.190	.215	.248	.259

Table XI.

Water as it enters the tank - and after standing at room temperature for seven and fourteen days.

	Original	After 7 days	After 14 days.
Free ammonia	11.84	26.40	25.6
	12.06	26.80	26.0
Alb. ammonia	.80	.45	.96
	1.20	.40	.96
Total Organic Nitorgen.	6.00	5.40	5.0
	6.20	5.2	4.8
Nitrites	.562	trace	trace
	.625	trace	trace
Nitrates.	2.095	.72	.32
	2.238	.64	.34

The medium used in tabl s IX and X is not identical with that used in table XI, the former having been heated and boiled would consequently change it chemi ally. The water had to be dilute[illegible] twenty times before any distillations were made with a slight error in Nessler[illegible] results given may not be entirely free from errors.

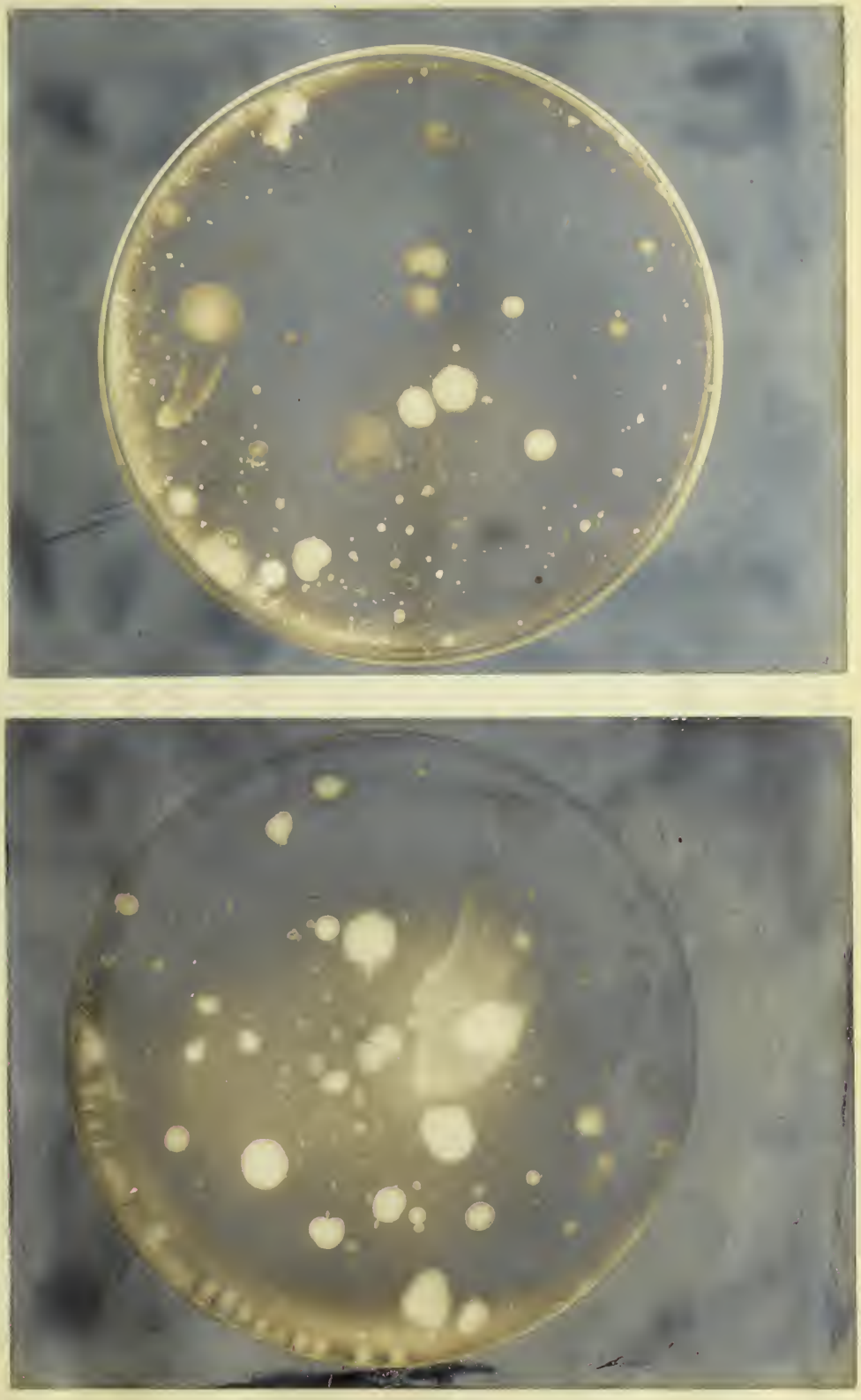
From tables IX and X we see that only slight changes are produced by the individual species. In most of the cases the greatest change takes place during the first seven days after innoculation; this is also true in the case where they are all acting in common as shown in table XI.

Thus the millions of bacteria brought into this tank from the various sources find a medium which contains the required constituents for their life and growth. The putrifactive ones have the power to form gases and these either remain dissolved or escape into the air. Not all species form the same gases, nor do the same species form similar gases under different conditions. The water as it enters the tank remains in the first section long enough to deposit most of its solid organic matter. It contains the largest number of bacteria and consequently the greatest change occurs here. As the water passes on carrying the undecomposed remnant of the organic matter, this gradually becomes oxidized and the number of organisms is greatly reduced. Very slight changes are produced by the individual species as compared with the case when all act in common.

The oxidation of the organic matter is due to these bacteria and not to sedimentation, for very little organic matter is found in the sludge. The greatest work done seems to be attributable not to one single species but several different kinds, most prominent among these as near as can now be ascertained are Bacillus coli-communis, Bacterium aerogenes, Bacillus vulgaris, and the one herein numbered XIV. The temperature of the tank at any time does not inhibit the growth of any of these; all of them grow within the limits of 10 - 37 degrees centigrade; all however grow better at room temperature or higher. The first three form gases of very similar proportion of carbonic acid gas (CO_2) and hydrogen (H), the CO_2 varying from 22 to 28 per cent. and the hydrogen from 68.6 to 75 per cent. approximately a ratio of 1 to 3. Bacillus vulgaris besides these, forms a small per cent. of marsh gas (CH_4). Bacillus coli-communis causes a decrease in the free ammonia, total organic nitrogen, nitrites and nitrates and an increase in the albumenoid ammonia amounting to the loss of the other constituents. Bacterium aerogenes produces an increase in the total organic nitrogen, after seven days; a decrease in the nitrites, with the other parts remaining practically unchanged. Bacillus XIV does not form gases, yet it appears to be one of the most active in oxidizing the organic matter. It is a rapid grower forming a heavy membraneous surface growth, and is a strong liquifier; it increases the amount of free ammonia, while there is a decrease in the albuminoid ammonia, organic nitrogen, nitric acid nitrates.

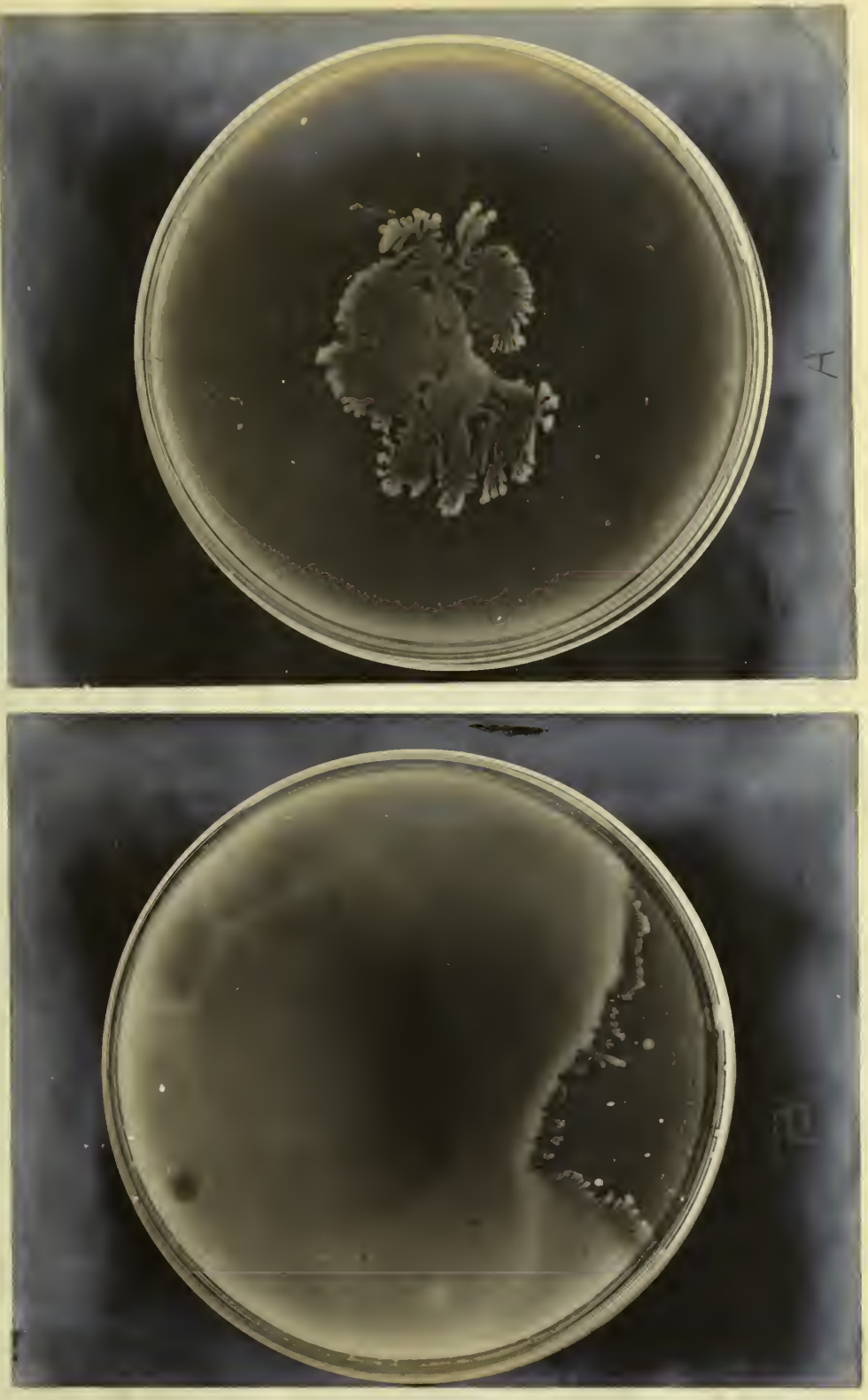
All except the last kind appeared in every sample; Bacillus coli-communis and Bacterium aerogenes could be easily detected on lactose-litimus plates, and occurred in large numbers in every sample; Bacillus vulgaris also occurred on every plate and on an average about three, thus making an approximate number of 30,000 per cubic centimeter. Bacillus XIV was not so abundant; it was found on about one plate out of ten, thus making a rough average of one thousand per cubic centimeter.

Plate I.



Plates showing colonies from 1/10000 c.c. of sewer water.

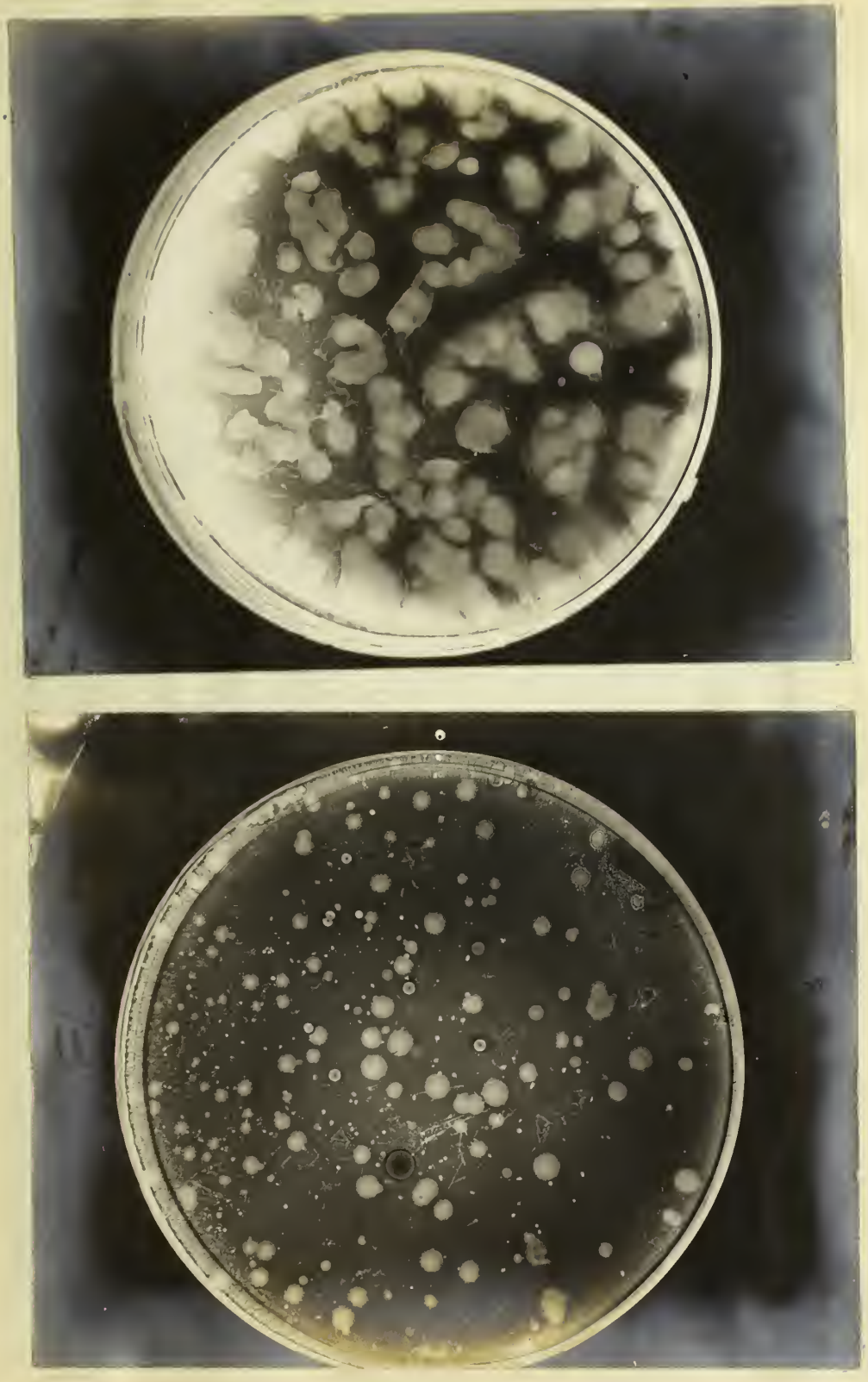
Plate II.



(1) A pure culture of Bacillus ------- VIV.

(2). Bacillus ----- VIV as it spreads when it occurs with others in the water.

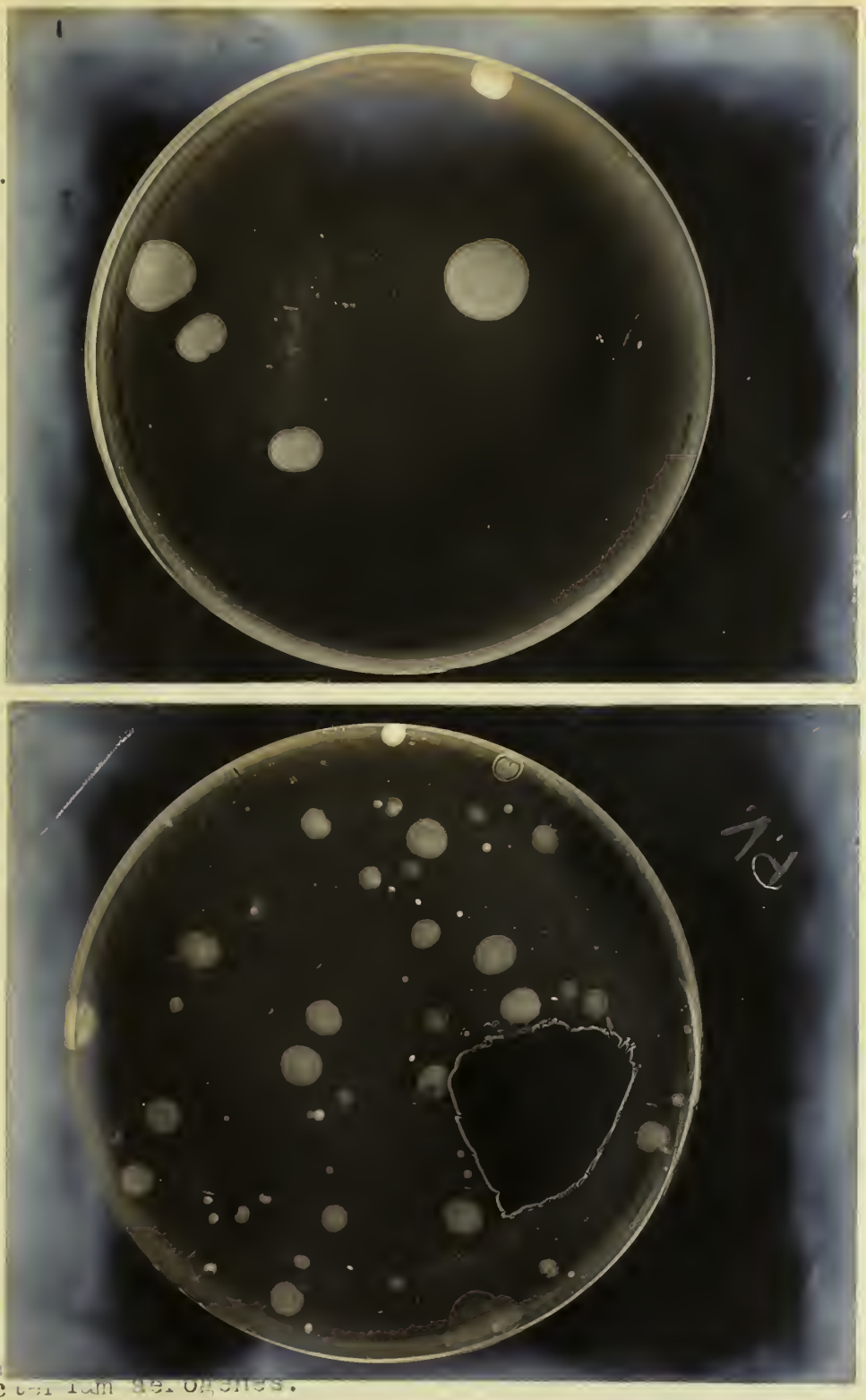
Plate III.



(1). Bacillus mycoides, showing its liquefaction on gelatin.

(2). Bacillus coli-communis. A few air bubbles give the appearance of contamination.

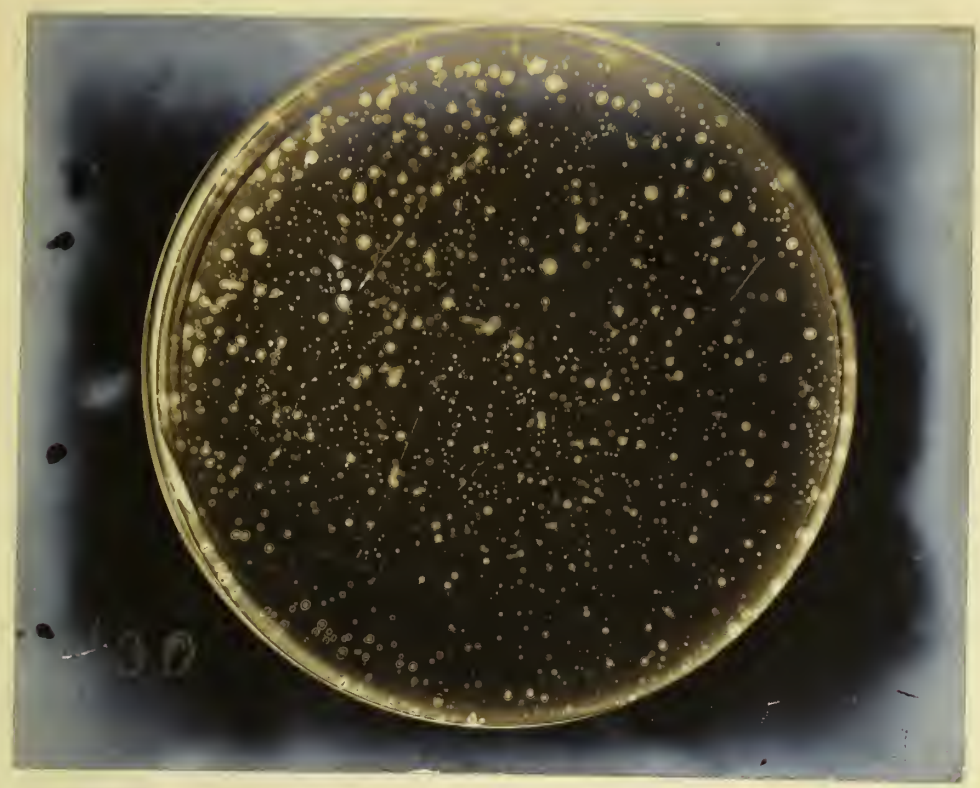
Plate IV.

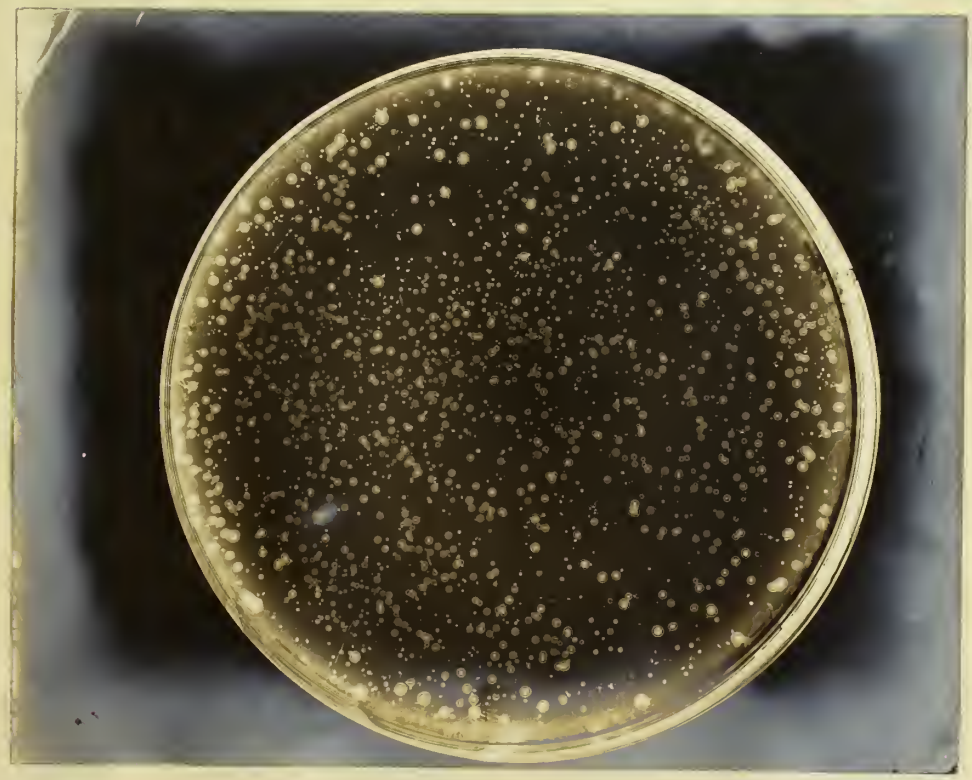


(1). Bacterium aeruginosa.

(2). Bacillus vulgaris.

Plate V.





(1). Bacillus ---------- XLI.

(2). Bacterium album.

BIBLIOGRAPHY.

Dyar, H.: On certain bacteria from the air: Annals of N.Y. Academy of Science **8**: 322.

Smith, Theobald: Wilder Quarter Century: 1868-1893.

Dr. McWeeney: Modern medicine and bacteriology review **3**: Aug. 1894.

Rideal: Sewage.

Berger: Uber Ptomaine.

Bennett, A.A. and Pammel, F.E.: Study of some gas producing bacteria: Journal of American chemical society **18**,157. 1896.

Mason, W.P.: Chem. v.s. Bacteriological examination: Journal of American Chemical Society.

Peckham, A.W.: Influence of environment upon the biological processes of the various members of the colon group.

Baker, W.W.: Sewage and sewage purification.

Wright, J.H.: Report on the results of an examination of the water supply of Phil.: Memoirs of the National Academy of Science **7**: 421 - 467. 1894.

Billings, J.S.: The influence of light upon the bacillus of Typhoid and the Colon Bacillus: Memoirs of the National Academy of Sciences **7**: 479-482, 1894.

Engler and Prantl: Die natürtichen Pflanzenfamilien 129: 1895.

Fuller, Geo.W. and Johnson, Geo. A. : On the differentiation and Classification of Water Bacteria: Journal of Experimental Medicine **4**: 609.

Sternberg: Manual of Bacteriology.

Newman: Bacteriology.

Migula: System der Bakterien **1** & **2**.

Journal of American public health Association 405. 1895.

Report of Del. College of Agricultural Ex. Station: 1897.

Mass. State Board of Health: 329. 1890.

" " " " " : 395-421. 1891.

" " " " " : 591. 1895.

" " " " " : 1897 & 1899.

Eisenberg: Bakterioloische Diagnostik.

Frankland, P. and G.C.: Micro-organisms in water, 1894.

Novy: Laboratory work in Bacteriology, 1899.

Sutton: Volumtric Analysis: 522-528.

Hempel: Gas Analysis.

Talbot, A.N.: Champaign Septic Tank: Engineering News: Aug. 17, 1899.